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Dynamics of Native Indian Food Fisheries on Salmon in British Columbia

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PETERMAN, R. M. 1980. Dynamics of native Indian food fisheries on salmon in British Columbia. Can. J. Fish. Aquat. Sci. 37: 561–566.

Basic components of predation processes are briefly reviewed and data on these components are presented for several native Indian food fisheries. Depensatory mortality emerges as a common effect of these fisheries, which supports findings of earlier workers on Skeena River sockeye salmon (*Oncorhynchus nerka*). Native food fisheries therefore operate like natural "Type II" predators, and descriptive equations are provided for use by salmon managers and modelers.

Key words: fishermen as predators, native, Indian, food, fisheries, salmon, depensation

PETERMAN, R. M. 1980. Dynamics of native Indian food fisheries on salmon in British Columbia. Can. J. Fish. Aquat. Sci. 37: 561–566.

L'auteur fait une brève revue des éléments de base de la prédation et présente, pour plusieurs pêcheries de subsistance pratiquées par les Indians, des données sur ces éléments. Comme effet commun de ces pêcheries, émerge une mortalité anticompensatoire, ce qui confirme les observations de chercheurs qui avaient déjà étudié le saumon nerka (*Oncorhynchus nerka*) de la rivière Skeena. Les pêcheries de subsistance des Indiens agissent donc comme prédateurs naturels de «type II», et on donne des équations descriptives pouvant être utilisées pour la gestion et l'élaboration de modèles de pêcheries de saumons.

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NATIVE Indian food fisheries for salmon (*Oncorhynchus* spp.) are important in British Columbia. Many communities have traditionally relied heavily on food catches of salmon, and the Federal government, as part of the salmonid enhancement program, is attempting to evaluate the impact of various enhancement schemes on native peoples. Also, harvest of salmon by native Indians most often occurs upstream as salmon approach spawning grounds. Because Indians are last in line to get a share of the catch, regulation of these fisheries is the last chance for fisheries managers to manipulate escapement levels.

Since 1955, reported food fishery catches in B.C. have ranged from 240 000 to 500 000 salmonids, of which 47 to 70% have been sockeye salmon (*O. nerka*) (McKay 1977). These catches compare with escapement figures for B.C. sockeye and total salmonids of 1–5.2 and 6–17 million fish, respectively. What is not obvious from these total figures is that food fisheries

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can, in specific cases, take a significant portion (>25%) of the fish that are in the river at the point where they are harvested. Several studies in the past have pointed out that food fisheries exert a depensatory mortality effect (Neave's 1953 term); in other words, a higher percentage of the fish is caught at low than at high fish abundance (Larkin and MacDonald 1968; McKay 1977). If common, this pattern is of concern to managers because it means that there is greater danger of underescapement in years of very poor total adult returns.

For these reasons it is important to understand the relation between food fisheries catches and abundance of fish. This paper analyzes responses of native food fisheries to fish abundance, using data from several areas in B.C. Commercial harvesting of salmon by native Indians is excluded from the present analysis; we are only concerned here with freshwater catches taken for food by Indians. The methods traditionally used for this harvest include dip nets, gaffs for hooking fish in pools, gill nets, and various trap mechanisms.

Fishermen as Predators

Various processes which occur in natural predator–prey relations have either been documented for, or included in, models of fishermen–fish interactions. Examples are competition (as implied by the standard exponential catch equation) and cooperation (Rothschild 1977), aggregation of boats in response to certain measures of fish abundance (Loucks and Sutcliffe 1978; Hilborn and Ledbetter 1979; Peterman et al. 1979), and changes in searching efficiency in relation to fish abundance (Paloheimo 1971; Paloheimo and Dickie 1964). With the exception of Ward and Larkin (1964) and Larkin and MacDonald (1968), few studies have attempted to determine the type of mortality (compensatory or depensatory) that results from the combination of these processes in human fisheries.

Here the comparison between Indian fishermen and natural predators will focus on the relation between percentage of food fishing mortality and abundance of fish, using annual data. There are three major components to this relation: the functional, numerical, and competition responses (Solomon 1949; Holling 1959). The functional response is the relation between number of fish consumed per predator per unit time and fish abundance. It reflects the change in efficiency with which prey are captured as fish abundance changes. Four basic shapes of functional responses have been identified and each is associated with a unique relation between prey numbers and percent mortality per predator (Holling 1959). Examples of three types are shown in Fig. 1. The numerical response shows how predator abundance varies with prey numbers, in particular here because of short-term aggregation responses. Finally, competition can occur among predators either directly through crowding or indirectly through depletion of the prey population. These processes are more thoroughly reviewed in a fisheries context by Peterman and Gatto (1978) and Larkin (1979).

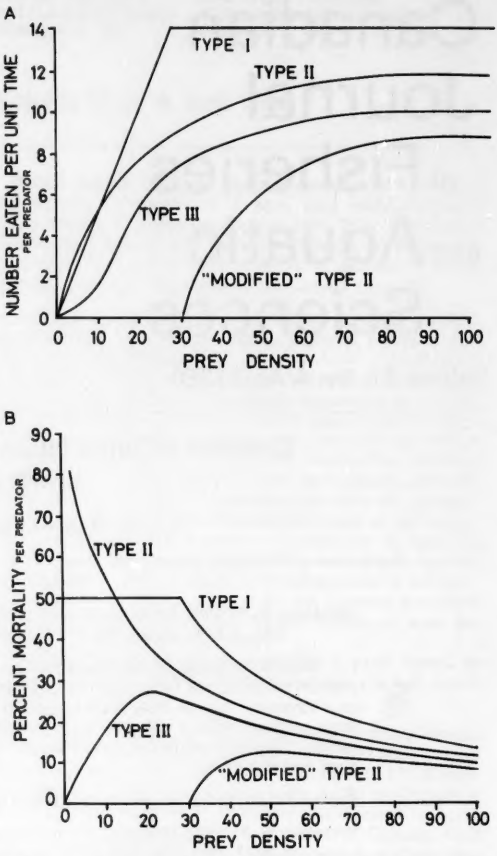

FIG. 1. Shapes of different types of functional responses (A) and their corresponding percent mortality curves (B). Reprinted with permission from Peterman and Gatto (1978).

Methods

Sources of Data

The relationships described above for natural predators were examined for Indian food fisheries on several stocks of salmon in B.C., using data from 1947 through 1976. Annual catches and escapements for Skeena River fisheries were provided by Zyblut (E. Zyblut, Fisheries and Marine Service, Vancouver, B.C., 1976, personal communication). Fraser River sockeye food fishery catches, escapements, and permit data are from International Pacific Salmon Fisheries Commission (IPSFC) Annual Reports,

Ricker and Manzer (1974), and McKay (1977). Annual catch data represent the reported freshwater catch only; illegal catch by native Indians is an unknown quantity and is excluded from this analysis. Fishing effort is expressed as number of permits issued per year, with usually one permit going to each family head even if several family members fish. Recently, permits have been issued to band councils instead (such as the band that harvests Birkenhead River sockeye) (McKay 1977). For the years discussed here, the major regulation on Indian food fisheries has been area closures, aimed at obtaining sufficient escapements.

Total numbers of fish available in the river were estimated by adding annual upriver escapements to reported annual Indian catches. This method assumes that there is no significant mortality agent (such as sportfishing) upriver between harvest areas and spawning grounds. Cases were carefully chosen to ensure that this assumption was met. Total escapements were used for all systems except the Harrison–Birkenhead, where jacks were subtracted from totals because the gill nets used there do not catch these immature adults (J. Woodie, International Pacific Salmon Fisheries Commission, personal communication). The size of the pool of fish which was to be harvested was then related to fishing effort, catch, and percent mortalities to see if regular, predictable patterns appeared which were analogous to those of natural predators.

Types of Responses

The numerical response was examined very simply. Effort levels were regressed on fish abundance of that same year, and separately on abundance of the previous year to determine if there was an aggregation response of fishermen.

The form of the functional response was identified by examining plots of the data and by fitting with a nonlinear least-squares procedure a generalized predation equation (C. S. Holling, University of British Columbia, Vancouver, B.C., 1975, personal communication):

$$y = (a \cdot X)/([b \cdot \exp(c \cdot X)] + X)$$

where X is fish abundance, y is catch, and a, b, and c are parameters. Certain parameter conditions lead to a Type III response ($c > 1/b$) and others ($c = 0$ and $< 1/b$) indicate a Type II. For cases where a Type II response was indicated, another equation with more biologically meaningful parameters was used. This equation is the Michaelis–Menten [$y = a \cdot X/(b + X)$], and its parameters were also estimated by a nonlinear, least-squares method. The parameter "a" represents the maximum catch and "b" is the fish abundance which results in half the maximum catch.

Results

Numerical Response

Effort data were only available for sockeye salmon (*O. nerka*) food fisheries on the Harrison–Birkenhead, Chilcotin, Stuart, Nechako, and North Thompson rivers. In no case was there a tendency for any change in effort with changes in fish available at the location where harvesting is done ($P > 0.1$, all sample sizes >17). This is true for both regressions on present year abundance and previous year numbers. In other words, effort seems to be independent of fish abundance. There are two cautions in drawing this conclusion. First, there could actually be more members of each family fishing in years of greater fish abundance, and this would not be reflected by number of permits which have been until recently one per family. Second, the amount of time spent fishing per individual varies but is not accounted for by number of permits issued.

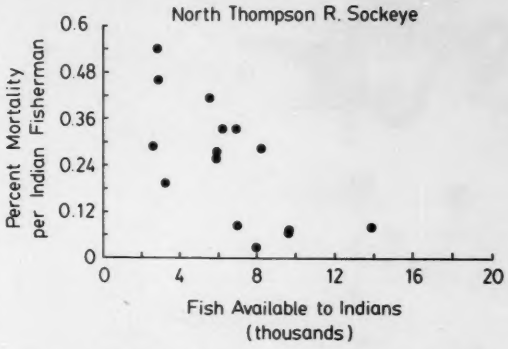

FIG. 2. Percent mortality per permit as a function of fish abundance for food fishery on North Thompson River sockeye salmon.

Functional Responses

Any reporting errors would make it difficult to distinguish between the different types of functional responses if we only examine plots of catch per effort as a function of prey abundance (see Fig. 1A). However, the distinction is more easily made if we plot percent mortality per unit effort as a function of fish abundance (Fig. 1B). When this is done, four of the five rivers show clear depensatory mortality; i.e., higher mortality per unit effort at low than at high fish abundance (example in Fig. 2). These four cases are sockeye fisheries on the Chilcotin, Nechako, North Thompson, and Stuart rivers. This monotonically decreasing percent mortality per unit effort conforms to Neave's (1953) depensatory mortality and the so-called Type II functional response (Holling 1959). This type of response is most clearly illustrated by the catch per effort curve for the Stuart River sockeye fishery (Fig. 3).

Parameter values estimated for the Holling generalized predation equation support the above conclusion that a Type II response is the best explanation of most of the data. In no case was there the parameter condition which indicates a Type III response. Data for the five species caught on the Skeena, and sockeye of the Nechako and Harrison–Birkenhead rivers are widely scattered, or measured over a relatively narrow range of fish abundances; for these cases *all* types of responses fit equally poorly. But in the other stocks, Type II responses were clearly indicated.

It should be noted that there is some concern that

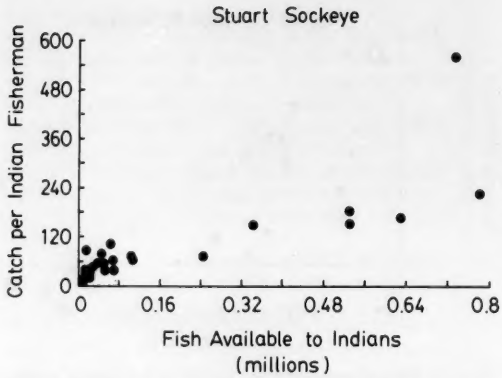

FIG. 3. Indian food catch per permit in relation to fish abundance for Stuart River sockeye salmon.

recent years of high salmon prices may have resulted in higher-than-normal catches (J. Woodie, IPSFC, 1977, personal communication). However, fishing mortality rates since 1973 have *not* usually been higher, *for a given abundance of fish in the river*, than in previous years. Hence the present analyses are not biased upward. The effect of higher prices on unreported catch is another matter, outside the scope of this paper.

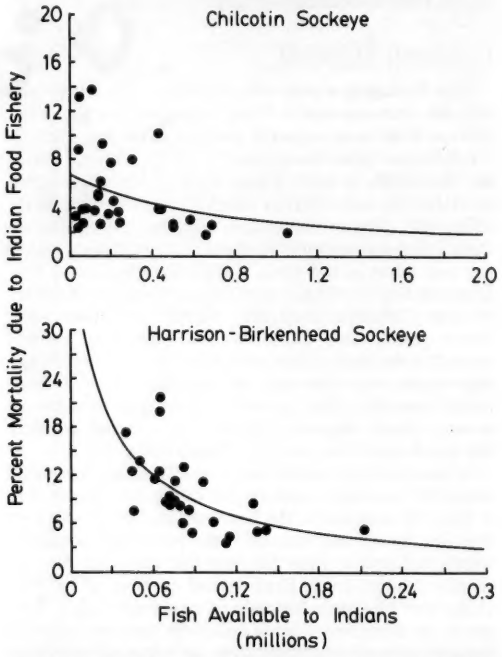

FIG. 4. Total percent mortality from Indian food fisheries as a function of fish abundance for Chilcotin and Harrison–Birkenhead rivers sockeye.

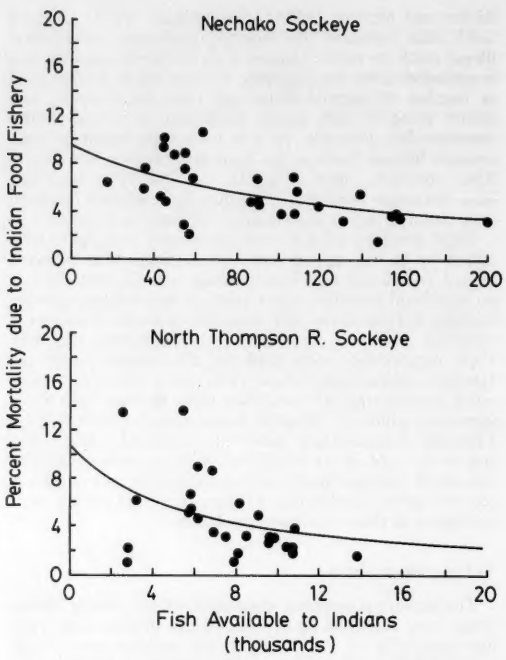

FIG. 5. Total percent mortality from Indian food fisheries as a function of fish abundance for Nechako and North Thompson rivers sockeye.

TOTAL RESPONSE

The product of effort levels and functional responses gives the total response of the Indian food fisheries. Data for several stocks in Fig. 4–6 show the depensatory nature of this harvesting process. Most striking is the relation between percent mortality and fish abundance for the total Fraser River sockeye (Fig. 6B); there is a surprisingly good fit despite variance contributed by occasional closures. Table 1 lists parameter values for the form of Michaelis–Menten equation used to describe these relations, plus the sum of squared deviations of data from this relation, and sum of squared deviations from fitting just the arithmetic mean. Formal F-tests cannot be used to compare these sums of squares because one parameter enters nonlinearly in the Michaelis–Menten equation. However, any reduction in sums of squares is useful from a management point of view, because predictability is improved.

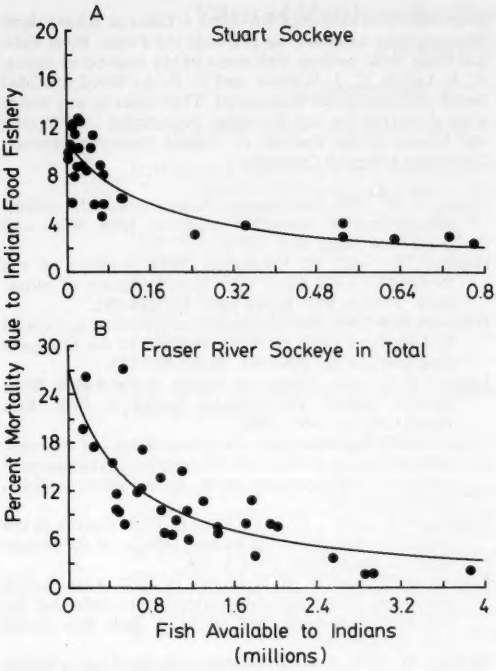

FIG. 6. Total percent mortality from Indian food fisheries as a function of fish abundance for Stuart River (A) sockeye and total Fraser River system sockeye (B).

Discussion

Findings in this study show that many Indian food fisheries act as depensatory mortality agents, as shown earlier for two sockeye fisheries by Larkin and MacDonald (1968). However, it cannot be assumed that these food fisheries take essentially a constant catch. Figure 7 shows that for the Stuart sockeye fishery, catches do drop off as fish abundance decreases, despite the fact that percent mortalities increase.

TABLE 1. Parameter values and sums of squares for the relation between total percent mortality due to Indian food fisheries and fish available, corresponding to Fig. 4–6. The equation for this relation is $Y = a \cdot 100/(b + X)$, where Y is in percent mortality and X is number of fish available. SS $\bar{x}$ is sum of squares for fitting with the mean percentage of mortality and SS equation is for fitting with the above relation. Skeena stocks are omitted because they do not clearly show the depensatory mortality pattern.

Species	River	a	b	SS $\bar{x}$	SS eq.
Sockeye	Chilcotin	47575.4	715988.0	298.0	262.0
Sockeye	Harrison–Birkenhead	9860.3	23076.0	566.0	340.0
Sockeye	Nechako	10949.0	117080.0	159.0	112.0
Sockeye	North Thompson	577.5	5314.9	284.0	236.0
Sockeye	Stuart	18775.0	175520.0	321.0	102.0
Sockeye	Fraser (total system)	149988.0	550208.0	1187.0	472.0

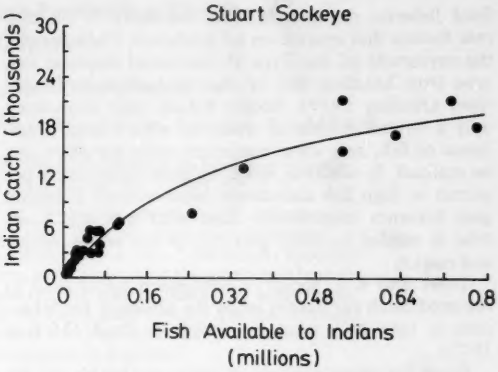

FIG. 7. Total Indian food fishery catches of Stuart River sockeye in relation to fish abundance.

Relations such as the ones shown here are potentially useful for salmon managers because percent removals by food fisheries can be reasonably well predicted, if numbers of fish escaping past commercial fisheries can be estimated from test fisheries. Desired escapements can therefore be more accurately achieved.

Relatively large percentages of fish available in the river can be removed by food fisheries at low fish abundance: 27% for Fraser sockeye, 17% for each of Skeena chinook, chum, and coho salmon. These significant precentages can help to keep small or declining runs down and affect their population dynamics, to the point of helping to maintain cyclic dominance (Ward and Larkin 1964; Larkin 1971).

The shapes of the observed relations are interesting from a theoretical viewpoint as well. The close correspondence between the form of some natural predator functional responses and those found in many Indian

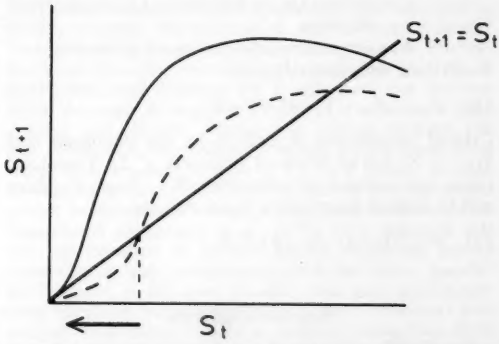

FIG. 8. Schematic representation of noncritical (solid line) and critical (broken line) depensation in plot of spawners (S) in generation $t + 1$ as a function of spawners in the previous generation.

food fisheries reemphasizes the similarity of limiting rate factors that operate on all predators. For example, the asymptote of the Type II functional response can arise from handling time or searching efficiency limitations (Holling 1959). Indian fishing gear can sweep only a limited volume of water, so with a large abundance of fish, only some maximum catch per effort can be realized. In addition, handling time limits catch per permit at high fish abundance because even if fishing gear becomes immediately filled with fish, significant time is needed to bring gear out of the water, empty and reset it.

There also is a "socially" determined upper limit to reported catch per permit set by the tendency for fishermen to take only what is needed for food (McKay 1977).

From the above analysis, it seems reasonable for the purposes of modeling salmon population dynamics to include the effect of Indian food fisheries as a depensatory mortality process. *At a minimum*, this will create what Clark (1976) calls noncritical depensation, where there is an S-shaped ascending limb on the relation between parents and progeny surviving to spawn (Fig. 8). This relation results in a slower recovery from heavy exploitation than would be the case if the stock behaved according to the standard Ricker model.

Critical depensation (Clark 1976) is the term given to the situation where the broken curve in Fig. 8 crosses below replacement levels at low stock sizes. That is, there is a critical stock size below which the population tends to move toward extinction. Fishery managers can determine if they are operating in this critical depensation mode by applying the simple guideline shown below in eq. 2.

Let

S_t = spawners in generation t

R_{t+1} = recruits to commercial fishery (preseason forecast), resulting from S_t

F = fish available to Indians

a = proportion of R harvested by commercial fisheries

b = proportion of F harvested by Indians.

From these definitions we have

$$(1) \quad S_{t+1} = R_{t+1} \cdot (1 - a) \cdot (1 - b).$$

Critical depensation is defined by the condition that $S_{t+1} < S_t$ for all levels of spawners $\leq S_t$. Therefore, using any method of estimating R_{t+1} from S_t, there will be critical depensation if, on average,

$$(2) \quad R_{t+1} \cdot (1 - a) \cdot (1 - b) < S_t.$$

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Jim Woodie (IPSFC) provided useful detailed information on Fraser River fisheries, as did Les Goodman of Canadian Department of Fisheries and Environment. Ed Zyblut generously provided data for Area 4 (Skeena River) food fisheries, Max Ledbetter helped code the Fraser River data, and Greg Steer assisted with some of the final curve fitting. P. A. Larkin, C. J. Walters, and F. E. A. Wood provided useful criticism of the manuscript. This research was begun while I worked for the Canadian Department of Fisheries and Oceans at the Institute of Animal Resource Ecology, University of British Columbia.

Clark, C. W. 1976. Mathematical bioeconomics: the optimal management of renewable resources. John Wiley and Sons, New York, N.Y. 352 p.

Hilborn, R., and M. Ledbetter. 1979. Analysis of the British Columbia purse seine fleet: dynamics of movement. J. Fish. Res. Board Can. 36: 384–391.

Holling, C. S. 1959. The components of predation as revealed by a study of small mammal predation of the European pine sawfly. Can. Entomol. 91(5): 293–320.

Larkin, P. A. 1971. Simulation studies of the Adams River sockeye salmon (*Oncorhynchus nerka*). J. Fish. Res. Board Can. 28: 1493–1502.

1979. Predator–prey relations in fishes: an overview of the theory, p. 13–22. *In* H. Clepper [ed.] Predator–prey systems in fisheries management. Sport Fishing Institute, Washington, D.C.

Larkin, P. A., and J. G. MacDonald. 1968. Factors in the population biology of the sockeye salmon of the Skeena River. J. Anim. Ecol. 37: 229–258.

Loucks, R. H., and W. H. Sutcliffe Jr. 1978. A simple fish population model including environmental influence, for two western Atlantic shelf stocks. J. Fish. Res. Board Can. 35: 279–285.

McKay, W. 1977. A socioeconomic analysis of native Indian participation in the B.C. salmon fishery with the proposed salmonid enhancement program. Fish. Environ. Can. PAC/T-77-8: 90 p.

Neave, F. 1953. Principles affecting the size of pink and chum salmon populations in British Columbia. J. Fish. Res. Board Can. 9: 450–491.

Paloheimo, J. E. 1971. A stochastic theory of search: implications for predator–prey relations. Math. Biosci. 12: 105–132.

Paloheimo, J. E., and L. M. Dickie. 1964. Abundance and fishing success. Rapp. P-V. Reun. Cons. Perm. Int. Explor. Mer 155: 152–163.

Peterman, R. M., and M. Gatto. 1978. Estimation of functional responses of predators on juvenile salmon. J. Fish. Res. Board Can. 35: 797–808.

Peterman, R. M., W. C. Clark, and C. S. Holling. 1979. The dynamics of resilience: shifting stability domains in fish and insect systems. *In* R. M. Anderson, B. Turner, and R. E. Taylor [ed.] Population dynamics. Blackwell Sci. Publ., Oxford. (In press)

Ricker, W. E., and J. I. Manzer. 1974. 1. Recent information on salmon stocks in British Columbia. Int. North Pac. Fish. Comm. Bull. 29: 1–24.

Rothschild, B. J. 1977. Fishing effort, p. 96–115. *In* J. A. Gulland [ed.] Fish population dynamics. John Wiley and Sons, London.

Solomon, M. E. 1949. The natural control of animal populations. J. Anim. Ecol. 18: 1–35.

Ward, F. J., and P. A. Larkin. 1964. Cyclic dominance in Adams River sockeye salmon. Int. Pac. Salmon Fish. Comm., Prog. Rep. 11: 114 p.

Effect of Metals on the Biomass Production Kinetics of Freshwater Copepods

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BORGMANN, U., R. COVE, AND C. LOVERIDGE. 1980. Effect of metals on the biomass production kinetics of freshwater copepods. Can. J. Fish. Aquat. Sci. 37: 567–575.

The effects of cadmium, copper, mercury, lead, and arsenic on the growth and mortality rates of a natural assemblage of copepods using natural food and water were determined. A seasonal cycle in toxicity was observed for all the metals except arsenic. Lead toxicity appeared to be related to the ash-free dry weight of the seston, but the reason for the variation in toxicity of cadmium, copper, and mercury remain unknown. The chronic toxicity of some metals differs much less between copepods and daphnids than does acute toxicity. The mathematical relationship between growth and mortality rates and metal concentration is discussed and a technique for calculating a sublethally toxic concentration is described.

Key words: copepods, heavy metals, biomass production, cadmium, copper, mercury, lead, arsenic, toxicity

BORGMANN, U., R. COVE, AND C. LOVERIDGE. 1980. Effect of metals on the biomass production kinetics of freshwater copepods. Can. J. Fish. Aquat. Sci. 37: 567–575.

Nous avons déterminé les effets du cadmium, cuivre, mercure, plomb et arsénic sur les taux de croissance et de mortalité chez un assemblage naturel de copépodes, utilisant une eau et une nourriture naturelles. Pour tous les métaux, sauf l'arsénic, on observe un cycle saisonnier de toxicité. La toxicité du plomb semble liée au poids sec sans cendres du seston, mais on ne peut expliquer la variation de toxicité du cadmium, cuivre et mercure. La toxicité chronique de certains métaux diffère beaucoup moins entre copépodes et daphnides que la toxicité aiguë. Nous analysons la relation mathématique entre taux de croissance et concentration des métaux, et décrivons une méthode de calcul de la concentration de toxicité sublétale.

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PRIMARY production can be measured by commonly used procedures which permit not only in situ measurements, but also laboratory toxicity experiments using both artificial culture media and natural lake water (e.g. Wong et al. 1978). However, common methods for measuring secondary production in the field, such as for example, by determining egg development times and egg abundance (Edmondson and Winberg 1971), are not usually directly usable in toxicity experiments. Sublethal toxicity to zooplankton is usually determined under controlled laboratory conditions using single species laboratory cultures of zooplankters and constant food concentrations (e.g. Biesinger and Christensen 1972). This makes extrapolation to field conditions difficult, where numerous factors might influence toxicity. To estimate how in situ production by copepods is affected by temperature and food, Borgmann et al. (1979) incubated nauplii from a natural population of copepods in the laboratory in natural water containing a natural food supply. Growth and mortality rates were obtained by size fractionation of the copepods using a series of nylon screens. The data described below were obtained using the same technique. The purpose of this study, however, was to estimate the effect of several metals on the kinetics of in situ copepod production rates in the Burlington Canal. Moreover, we describe a method for linearizing growth curves which permits linear regression analysis of production rates and permits calculation of a kinetically defined sublethally toxic concentration.

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Materials and Methods

The experimental procedure used has been described in detail previously (Borgmann et al. 1979). Copepod nauplii were collected by pouring surface water from the Burlington Canal through a pair of nested screens (64- and 102-μm mesh sizes) and retaining the intermediate size fraction. After thorough mixing, the nauplii in a subsample were counted and aliquots, estimated to contain between 100 and 150 nauplii, were added to enough 44-μm-filtred canal water to give a final volume of 2 L in a battery jar with an aluminum foil lid. The exact number of animals present cannot be determined without killing them; however, other experiments (Borgmann et al. 1979) have indicated that this concentration of animals permits maximum growth rates (i.e. animal concentration does not affect growth) during most of the year. Nauplii were used immediately after collection and incubated under static conditions with various metal concentrations as indicated blow. Two 40-W fluorescent light bulbs, ~80 cm above the jars, provided indirect illumination (due to the opaque lids on the jars) on a 12-h-light : 12-h-dark photoperiod. The species present consisted primarily of *Cyclops vernalis* and *C. bicuspidatus thomasi*, although some *Diaptomus* spp. were also seen.

TABLE 1. The concentration series used most often for each metal, background metal concentrations, and fraction of the metal recovered from the water after a 2-wk incubation period at 15°C.

Metal	Concentrations used	Background levels (mean and total range in $\mu g \cdot L^{-1}$)	Percentage of metal recovered
Cd	0, 5, 10, 20, 40, 80 $\mu g \cdot L^{-1}$	1.6(<0.5–3)	69
Cu	0, 10, 18, 32, 56, 100 $\mu g \cdot L^{-1}$	3.7(0.8–7)	48
Hg	0, 10, 18, 32, 56, 100 $\mu g \cdot L^{-1}$	1.1(<0.05–2)	80
Pb	0, 0.1, 0.32, 1, 3.2, 10 $mg \cdot L^{-1}$	4.0(1–7)	56
As	0, 0.26, 0.64, 1.6, 4, 10 $mg \cdot L^{-1}$	1.5(<0.1–10)	96

After a 2-wk incubation period at 15°C the copepods were filtered through a series of Nitex nylon screens (505-, 363-, 275-, 202-, 153-, 102-, and 64-μm pore size) and counted. The copepod biomass was estimated by multiplying the number in each size class by the mean relative volume per organism for that size class and summing the biomasses for all size classes. Relative volumes (which equal 362, 149, 59.8, 24.8, 10.3, 3.7, and 1.0) were obtained from the relative sizes of spheres which would be retained between the mesh sizes indicated above or between a mesh size of 505 and 656 μm (Borgmann et al. 1979). All individuals pass through 656-μm mesh netting. The mean size per individual (V) is equal to the total biomass divided by the total number present (N). The growth rate (g), mortality rate (m), and cohort biomass growth rate (k) were calculated assuming

$$(1) \quad V = V_o e^{gt}$$

$$(2) \quad N = N_o e^{-mt}$$

$$(3) \quad B = NV = B_o e^{kt}$$

where N_o, V_o, and B_o are the initial number, size, and biomass, respectively, and t is time in days. Borgmann et al. (1979) showed that growth was exponential during the 2-wk incubation period at 15°C, verifying eq. 1. Since growth is exponential, the total instantaneous biomass production rate per unit biomass is equal to g (Allen 1971). The cohort biomass growth rate (k), which is equal to $g - m$ (from eq. 1, 2, and 3), is the rate of increase in biomass in the absence of predation, and hence is equal to the portion of the total biomass production rate available to higher trophic levels. We have previously called $r = g - m$ the biomass production rate in a study on snail production (Borgmann et al. 1978). This is misleading because the *total* biomass production includes production due to dead organisms up to the time of their death, and hence is equal to g. We therefore refer to $g - m$ as the cohort biomass growth rate here, and we have also changed the notation from r to k to avoid any possible confusion with the instantaneous rate of population increase, not measured here but usually denoted by r.

A portion of the 44-μm-filtered water, which provided the sole food supply during the experiment, was filtered through prewashed, preashed, and preweighed Whatman GF/C glass fiber filters, followed by drying at 60°C, weighing, ashing at 500°C, and reweighing to obtain the total and ash-free dry weight of the seston. Lake Ontario water locally has a hardness (as $CaCO_3$) of about 139 $mg \cdot L^{-1}$ and alkalinity (as $CaCO_3$) of about 88 $mg \cdot L^{-1}$ (Borgmann et al. 1978). The pH varied slightly from one collection date to another and ranged from 7.6 to 8.8.

The metals were added at the beginning of the incubation period in the form of $Cd(NO_3)_2 \cdot 4H_2O$, $CuCl_2 \cdot 2H_2O$, $HgCl_2$, and $Pb(NO_3)_2$. Arsenic was added in one of two forms, as arsenate ($Na_2HAsO_4 \cdot 7H_2O$), or as arsenite ($NaAsO_2$). The medium was not changed or stirred during the incubation period. The concentrations of the metals tested were varied occasionally as attempts were made to find the optimal concentration range needed for toxicity tests. The most commonly used concentrations are summarized in Table 1, along with the background concentrations measured prior to metal addition. All calculations of toxicity are based on nominal and not on measured concentrations. Metal concentrations were occasionally measured, and Table 1 also shows the mean percent of the metals recovered from the water after the 2-wk incubation period. The fraction of the metal recovered was not clearly related to the metal concentrations. Presumably metal not recovered was adsorbed onto the container since the total metal concentration was measured. None of the metals, even at the highest concentrations used, changed the pH of the water by more than 0.2 pH units. Metal concentrations were determined by atomic absorption spectrophotometry by the Water Quality Branch of the Canada Centre for Inland Waters according to procedures outlined in the *Analytical Methods Manual* (Anon. 1974) for Cd, Cu, Hg, and Pb, and using the procedure of Goulden and Brooksbank (1974) for arsenic.

Theory

The relationship between toxicant concentration and the rate of mortality (m) or growth (g) is not linear, making direct analysis of m or g difficult. For mortality, a linear relationship can be obtained by subtracting the control mortality rate (m') from m to give a toxicant-induced mortality rate (m'').

$$(4) \quad m = m' + m''.$$

An example is shown in Fig. 1, where m, m', and m'' are plotted against lead concentration on a double-log scale. This is identical with the procedure used previously to explain the relationship between snail mortality and lead concentration (Borgmann et al. 1978). The relationship between g and the toxicant concentration is similar to m except that, instead of increasing, g decreases with increasing toxicant concentration. A toxicant-affected growth rate

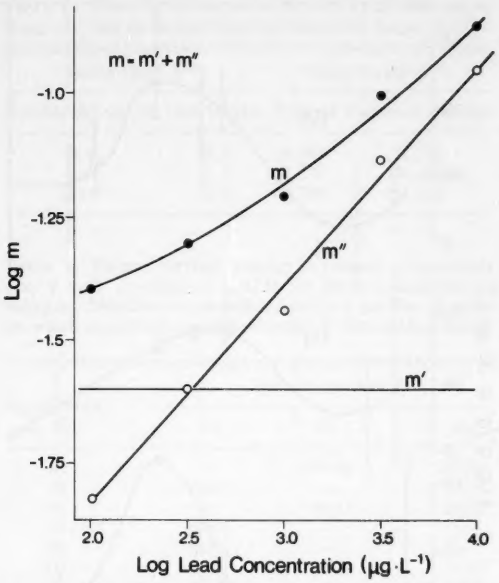

FIG. 1. The mortality rate (m), control mortality rate (m'), and toxicant induced mortality rate (m'') as a function of lead concentration on October 27, 1977.

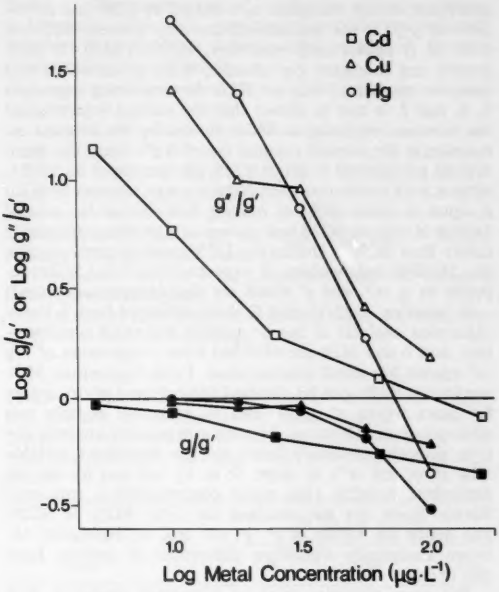

FIG. 2. The values of g/g' and g''/g' as a function of metal concentration for cadmium (August 25), copper (April 5), and mercury (June 6, 1978). g = growth rate, g' = control growth rate, and g'' = toxicant-related growth rate.

(g'') can be defined such that

$$(5) \quad (g)^{-1} = (g')^{-1} + (g'')^{-1}$$

$$(6) \quad g = \frac{g'g''}{g' + g''}.$$

Equation 5 is analogous to eq. 4, where g' represents the control growth rate. Because growth rate is the inverse of time required to reach a certain size, g'' is equivalent to the inverse of the toxicant-induced increase in this time. Some examples of g and g'' for cadmium, copper, and mercury are shown in Fig. 2, where g and g'' are divided by g' to correct for differences in control growth rates from one date to another. Both g'' and m'' produce approximately straight lines when plotted against metal concentration using log scales, and hence the relationship between g or m and metal concentration can be described using

$$(7) \quad g'' = CM^{-c}$$

$$(8) \quad m'' = BM^{b}$$

where M is the metal concentration, C and B are constants, and c and b are the absolute values of the slopes in Fig. 2 and 1, respectively.

The value of c for any given metal was not observed to vary significantly from one date to another, and hence the relative toxicity from day to day can be expressed as the ratio of g''/g' at a convenient intermediate concentration. A low value for g''/g' indicates high toxicity and a high g''/g' indicates low toxicity. Equations 4, 6, 7, and 8 are consistent in form with general equations derived for biological rates from theoretical considerations of the kinetics of multi-enzyme and membrane systems (Borgmann 1974).

Although toxicity can be adequately described using the c, b, and g''/g' values, it is common in toxicology to define toxicity as a concentration eliciting a certain response, such as for example, the LC50 (toxicant concentration lethal to 50% of the organisms in a specified time period). To make our data more comparable with other studies, a concentration will be defined and called the SC20, the sublethal concentration resulting in a drop in the cohort biomass growth rate k equal to 20% of the control growth rate ($0.2\,g'$). The SC20 is therefore the concentration where

$$(9) \quad g - m'' = 0.8\,g'.$$

Two other concentrations will also be defined; the G20 equal to the toxicant concentration where

$$(10) \quad g = 0.8\,g'$$

and the M20 equal to the toxicant concentration where

$$(11) \quad g' - m'' = 0.8\,g'.$$

Using equations 10 and 6, $g'' = 4\,g'$ at G20. Equation 11 gives $m'' = 0.2\,g'$ at M20. Since growth is exponential, the rate of biomass production per unit biomass is given by g (Allen 1971) and G20 is the toxicant concentration resulting in a 20% reduction in biomass production rate. M20 is the concentration resulting in a mortality which reduces the cohort biomass growth rate ($k = g - m$) by an amount

equivalent to the reduction in k caused by G20 (i.e. $m'' =$ 20% of g'). If the toxicant affects only growth, SC20 = G20. If it affects only mortality, SC20 = M20. If both growth and mortality are affected, SC20 is somewhat less than the smaller of G20 or M20. By combining equations 3, 4, and 9, it can be shown that the natural logarithm of the biomass remaining at SC20 divided by the biomass remaining in the control is equal to $-0.2\,g't$. Since the mean control growth rate is about 0.258 (Borgmann et al. 1979), after a 2-wk incubation period the average biomass at SC20 is equal to about 48%, or roughly half that of the control (which is why an SC20 was chosen as a measure of toxicity rather than SC50). Unlike the LC50 used in acute studies, the SC20 is independent of exposure time (i.e. SC20 depends on g, m'', and g' which are time-independent rates).

In practice, c, g''/g', and G20 are obtained from a linear regression analysis of log g'' against log metal concentration, and b and M20 are obtained from a regression of log m'' against log metal concentration. From regressions, 95% confidence limits can be obtained for c, b, and g''/g', except for cases where g'' or m'' can be estimated at only two concentrations. However, because a regression analysis can only provide confidence limits for the dependent variable (log g'' or log m'') or slope (c or b) and not for the independent variable (log metal concentration), true confidence limits are not obtained for G20, M20, or SC20, and hence the values of g''/g' are used to distinguish between statistically significant differences in toxicity from day to day.

For some purposes, such as ecosystem modeling, it is advantageous to be able to express biomass production rates and toxicant-induced mortality rates as a function of toxicant concentrations mathematically. For this purpose, the constants C and B in eq. 7 and 8 can be expressed in terms of G20 and M20. Since, as stated above, at G20, $g'' = 4\,g'$, and at M20, $m'' = 0.2\,g'$, the constants are given by $C = 4\,g'(\mathrm{G20})^{c}$ and $B = 0.2\,g'(\mathrm{M20})^{-b}$. Hence eq. 7 and 8 can be rewritten to give

$$(12)\quad g''/g' = 4\left(\frac{\mathrm{G20}}{M}\right)^{c}$$

$$(13)\quad m''/g' = 0.2\left(\frac{M}{\mathrm{M20}}\right)^{b}.$$

Equation 13 gives toxicant-induced mortality rates relative to the growth rate in the control. Growth rates in the presence of metals relative to control growth rates are given by rearrangement of eq. 6 to give

$$(14)\quad g/g' = \frac{g''/g'}{1 + g''/g'}$$

where g''/g' is obtained from eq. 12. Hence, an estimate of biomass production rates (= growth rates) and metal-induced mortality rates that would result from addition of any given metal concentration in the field can be obtained by multiplying the biomass production rates observed in the field by g/g' and m''/g', respectively, using equations 12, 13, and 14 and a knowledge of c, b, G20, and M20. This assumes, however, that production rates of large nongrowing members of the population (i.e. through reproduction) are similar to production of actively growing individuals.

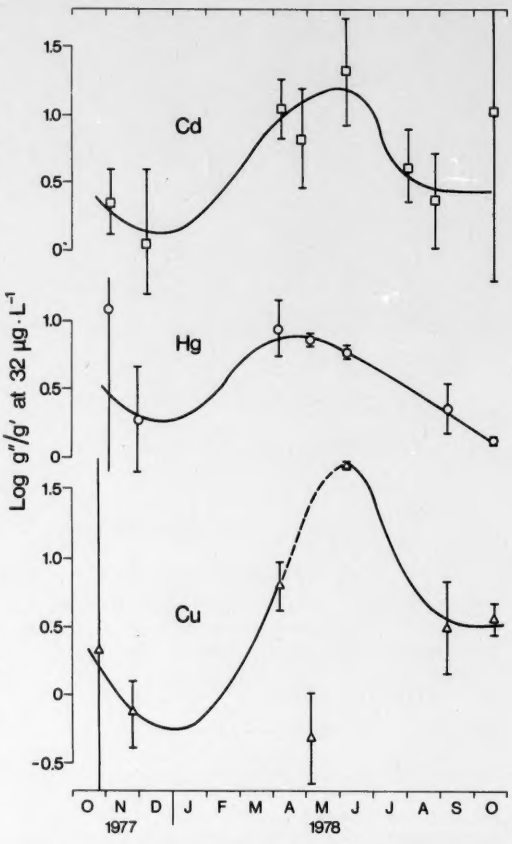

FIG. 3. The values of g''/g' at 32 $\mu g \cdot L^{-1}$, calculated from regression analysis of log g''/g' versus log metal concentration, as a function of the time of year for cadmium, copper, and mercury. Vertical bars indicate 95% confidence limits.

Results

CADMIUM, COPPER, AND MERCURY

In the presence of cadmium, copper, or mercury, growth was reduced at lower concentrations than those which increased mortality. Hence toxicity is primarily a function of g''. The ratio of g''/g', and its 95% confidence limits, obtained from regression analysis as described above, is shown in Fig. 3 for each of the three metals at 32 $\mu g \cdot L^{-1}$. (The value of g' ranged between 0.180 and 0.345 for all the toxicity experiments.) Each point in Fig. 3, and each value in Tables 5, 6, and 9 represent data collected from a single experiment (water and animals collected at the same time), between October 1977 and October 1978. With the ex-

TABLE 2. The effect of seston on the toxicity of cadmium at 80 $\mu g \cdot L^{-1}$ during an experiment conducted on August 3, 1978.

Seston (mg · L^{-1})		Growth rate (d^{-1})	
Ash-free dry weight	Ash weight	Control	Cadmium exposed
0.9	0.6	0.284	0.232
1.8	1.2	0.276	0.246
3.6	2.4	0.288	0.245

TABLE 3. Percent survival, relative to control, of copepods after a 2-wk exposure at 15°C to the metal concentrations indicated. Numbers in parentheses indicate number of dates on which experiments were performed at that concentration.

Metal concn. $\mu g \cdot L^{-1}$	Percent survival[a]		
	Cd	Cu	Hg
32	—	107(6)	107(6)
40	93(6)	—	—
56	—	95(5)	61(5)*
63	80(2)	—	—
80	81(6)	—	—
100	—	84(6)	29(6)*
160	46(2)*	—	0(1)
320	—	0(1)	—
400	27(1)	—	—

[a]*Significantly different from control at $P < 0.05$.

ception of one experiment in May using copper, each of the metals demonstrate a similar cycle in toxicity. Copepod growth appeared to be most sensitive to these metals in late fall and winter and most resistant in early summer. The reason for the yearly cycle in toxicity is unknown. Thermal shock is unlikely to contribute to this cycle since field temperatures (Borgmann et al. 1979) do not correlate well with toxicity. A large amount of scatter is present and no obvious relationship can be discerned if g''/g' is plotted against the ash-free dry weight of the seston. This is also true if the ash weight of the seston is examined. Hence, toxicity does not appear to be related to particulate matter in the water. Varying the seston by replacing part of the water in which the copepods were incubated with the same water after filtration through a glass fiber filter further demonstrates this (Table 2). The presence of 80 $\mu g \cdot L^{-1}$ of cadmium appears to reduce growth by an equivalent amount, regardless of the seston concentration.

The percent survival of copepods at various concentrations of cadmium, copper, or mercury is summarized in Table 3. Mercury significantly reduced survival at concentrations of 56 $\mu g \cdot L^{-1}$ or above, but cadmium or copper had little effect up to 80 or 100 $\mu g \cdot L^{-1}$. Since few concentrations provided estimates for m'', regression analysis of log m'' against log metal concentration was not possible and confidence limits for m'' could not be determined for individual dates. Hence seasonal variation in m'' was not examined. The values of c and b for the three metals are summarized in Table 4. Since the values of c did not vary significantly from one date to another, only the mean value is shown.

TABLE 4. The mean values of c and b for cadmium, copper, and mercury. The 95% confidence intervals are included. Numbers in parentheses indicate total number of separate estimates for c or b obtained.

Metal	c	b
Cd	0.78 ± 0.35 (4)[a]	1.22 ± 0.64 (1)[b]
Cu	1.62 ± 0.70 (7)	2.81 ± 1.23 (3)[c]
Hg	2.23 ± 0.30 (7)	2.65 ± 1.04 (5)[c]

[a]Estimates of c used only for dates when G20 < highest concentration tested (See Table 5).

[b]From data in Table 3 (95% confidence interval obtained from regression analysis).

[c]b calculated separately for each date when mortality was observed at both 56 snd 100 $\mu g \cdot L^{-1}$.

TABLE 5. The SC20 concentrations for cadmium, copper, and mercury. Underlined values indicate SC20 = G20. If mortality contributes to a decrease in cohort biomass growth rates near the G20s, the range within which SC20 must lie is given.

Date	SC20 ($\mu g \cdot L^{-1}$)		
	Cd	Cu[a]	Hg
Oct 25 – Nov 4, 1977	18	23	32–55
Nov 24 – Dec 7	5.7	20	31
April 3–5, 1978	>80	56–60	32–48
April 27 – May 4	~80	10–18*	32–43
June 6–7	>80	80	32–39
August 1	36	—	—
Aug 25 – Sept 7	17	27	25
October 18–19	>80	31	20

[a]*G20 from regression analysis is equal to 1.0, but no growth inhibition was observed at 10 $\mu g \cdot L^{-1}$ in the raw data.

The sublethally toxic concentration SC20 for each of the metals is shown in Table 5. In many cases no mortality was observed in the raw data at a concentration equal to (or the next concentration tested which was above) the G20, and hence SC20 is often equal to G20. The SC20 for cadmium varied considerably and on four occasions was greater than the highest concentration tested (80 $\mu g \cdot L^{-1}$). The SC20 values parallel the g''/g' values in Fig. 3 (see also eq. 12). The total variability in SC20, however, is also a function of c.

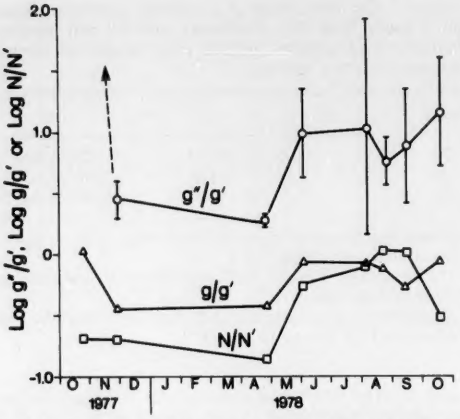

FIG. 4. The values of g''/g' at 1 mg·L^{-1}, calculated from regression analysis of log g''/g' versus log lead concentration, and the values of N/N' (fraction surviving relative to control) and g/g' obtained from the raw data at 10 mg·L^{-1} of lead as a function of the time of year. Vertical bars indicate 95% confidence limits.

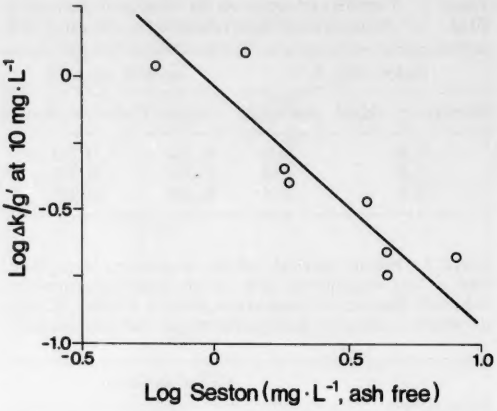

FIG. 5. The value of $\Delta k/g'$ ($\Delta k = g' - (g - m'')$) at 10 mg·L^{-1} of lead as a function of seston (ash-free dry weight). The line was obtained from regression analysis and is significantly different from 0 ($P < 0.01$, slope = −0.79).

Since c is large for mercury (Table 4), the G20 and therefore also the SC20 vary little for a given change in g''/g' whereas for cadmium, with the lowest c value, changes in g''/g' result in much larger variations in G20 (i.e. c is the slope for g''/g' in Fig. 2).

LEAD

The ratio of g''/g' for lead at 1 mg·L^{-1} as a function of the time of year is shown in Fig. 4. Although seasonal differences in toxicity are evident, the cycle is a little different from that of Cd, Cu, and Hg. Also shown in Fig. 4 are the ratios of g/g' and N/N' (N' = number surviving in control) obtained from the raw data at 10 mg·L^{-1}. Unlike Cd, Cu, or Hg, lead sometimes causes mortality even though the effect on growth is minimal (e.g., Oct. 1977 and Oct. 1978, Fig. 4). On other dates, however, toxicity is due primarily to growth inhibition (e.g. Sept. 1978). In Table 6 the values of c, b and SC20 obtained for each of the dates are summarized. Mortality was sufficient to obtain estimates of m'' at concentrations other than 10 mg·L^{-1} on only two occasions, both in October, one in 1977 and one in 1978. Data from October 27, 1977 are also shown in Fig. 1, which are the only data obtained in which b was small enough to provide a clear relationship between m'' and metal concentration. On all other dates b appears to be much larger although it could only be estimated again on October 20, 1978. Therefore b appears to be variable for lead, although the values of c are not significantly different from one another, and the SC20 may be equal to either the G20 or the M20, depending on the date of the experiment.

Since lead toxicity may be exerted through either g'' or m'', the ratio of g''/g' is less useful than for the other metals. Lead toxicity may be expressed as Δk ($\Delta k = (g' - m') - (g - m) = g' - (g - m'')$), although the relationship between Δk and lead concentration would not be expected to be linear on a double-log plot since eq. 4, 6, 7, and 8 cannot be combined to give a simple relationship such as eq. 7 or 8. The ratio of $\Delta k/g'$ at 10 mg·L^{-1}, obtained from the data in Fig. 4, is shown plotted against the ash-free seston in Fig. 5. The line has a slope of −0.79, which is significantly different from 0 at $P = 0.01$. Neither m'' nor g'' correlates with seston as well as does Δk. (Similar attempts to correlate Δk with seston for other metals were unsuccessful.) Lead, therefore, differs from Cd, Cu, and Hg, not only in the fact that mortality is important, but also because lead toxicity appears to be related to particulate matter in the water.

ARSENIC

Arsenic differs from the other toxicants studied here in that it is not a true metal and commonly occurs as anions (e.g. arsenate, arsenite) rather than cations. The toxicity of arsenate does not vary significantly from one date to another since all g''/g' confidence intervals overlap, although there is a possibility that toxicity is higher in April and May compared to March or October (see Table 9 below). Arsenate, therefore, does not show the seasonal cycles evident in the cationic metals previously discussed. Arsenite also shows no significant seasonal differences in g''/g'.

Since for both arsenate and arsenite m'' could be obtained at more than one concentration on only two

TABLE 6. The SC20 concentrations, and values of c and b for lead. Single underlined values indicate SC20 = G20 where G20 < M20. The double underlined value indicates SC20 = M20 where M20 < G20. On dates when growth was not significantly reduced and mortality occurred at only one or two concentrations, the range where SC20 must lie is given. The 95% confidence limits for c and b obtained from regression analyses of log g'' or log m'', respectively, against log lead concentration are included.

Date	SC20 (mg · L^{-1})	c	b
October 27, 1977	2.0	—	0.44±0.07
November 28	0.68	0.78±0.22	—
April 26, 1978	0.24	0.52±0.07	—
June 1	3.2–10	—	—
August 4	>10	—	—
August 23	>10	—	—
September 14	2.3	0.78±0.54	—
October 20	3.2–10	—	~1.2

TABLE 7. Percent survival, relative to control, of copepods after a 2-wk exposure at 15°C to the arsenic concentrations indicated. Numbers in parentheses indicate number of dates on which experiments were performed at that concentration.

Arsenic concn. mg · L^{-1}	Percent survival	
	Arsenate	Arsenite[a]
1.6	104(5)	100(4)
4.0	104(6)	50(5)*
10.0	74(5)	2(5)*

[a]*Significantly different from control at $P = 0.05$.

TABLE 8. The mean values of c for arsenic. The 95% confidence limits are included. Numbers in parentheses indicate total number of separate estimates for c obtained. For both arsenate and arsenite b could be obtained only twice, both estimates being shown.

Ionic form	c	b
Arsenate	0.91±0.74(5)	1.04–1.10
Arsenite	0.95±0.40(4)	1.8–3.0

dates, few estimates of b were obtained (Table 8). It is obvious, however, that b is much larger for arsenite than for arsenate, and this results in a more rapid increase in arsenite toxicity with increasing concentration compared with arsenate. On the other hand, c is similar for both forms of arsenic. The SC20 values are shown in Table 9. SC20 is equal to G20 for arsenate because of the low mortality. For arsenite, G20 is less than M20 on only one occasion, and SC20 is more a function of mortality than growth, although growth was

TABLE 9. The SC20 concentrations for arsenic. Single underlined values indicate SC20 = G20 where G20 < M20. Double underlined values indicate M20 where M20 < G20. If mortality or growth contribute to a decrease in cohort biomass growth rates below the G20 or M20, respectively, the range within which SC20 must lie is given.

Date	SC20 (mg · L^{-1})	
	Arsenate	Arsenite
October 24, 1977	—	0.32–1.0
March 14, 1978	3.61	—
April 14–25	1.38	1.6–3.2
May 29–30	1.49	4.0–7.2
September 12–14	3.78	1.6–4.6
October 19–20	3.47	1.6–4.2

usually less near the M20 concentration. Since m'' was not always obtainable at more than one concentration, the M20 values in Table 9 were calculated from the m'' at 4 mg·L^{-1} assuming a value of 2.0 for b. In general, arsenate has a similar or slightly higher toxicity than arsenite at low concentrations (<4 mg·L^{-1}) because of growth inhibition, whereas arsenite is the more toxic form at higher concentrations because of direct mortality (Table 7).

Discussion

The present study differs from most toxicological experiments because the data are analyzed in terms of the rates of growth and mortality, and probit analysis is not employed. This is the same approach that was used in a recent study on the effects of lead on snail production (Borgmann et al. 1978) where mortality was expressed as a rate to permit comparison with the growth rate and calculation of a lead concentration resulting in no net production. In the present study, however, growth was usually affected at a lower metal concentration than survival. Growth can be analyzed in an analogous fashion to mortality if calculations are based on the inverse of the growth rate. This results in additivity between $(g')^{-1}$ and $(g'')^{-1}$ where g'', the "toxicant affected growth rate," is linearly related to metal concentration on a log–log plot (eq. 5 and 7 and Fig. 2).

Since our studies were carried out using a natural population of copepods, natural water, and natural food supply, a considerable amount of variability was observed in metal toxicity. Although a certain drop in reproducibility of results occurs by not using a controlled laboratory procedure with single zooplankton and food species at constant concentration, the results obtained are more useful in helping to understand the effect of metals under natural conditions, where toxicity cannot be expected always to remain constant. For cadmium, copper, mercury, and lead a statistically significant difference in toxicity was observed between

some dates and others (Fig. 3 and 4). In fact, the only metal which did not demonstrate a seasonal variation in toxicity in this study was arsenic. This may be because arsenic, which is not a true metal, is commonly bound to oxygen in the form of an anion, rather than existing singly as a cation.

In general, toxicity was usually indicated by a drop in growth rate, rather than by increased mortality. The only exceptions were the mortality due to arsenite and, on some dates, the mortality due to lead, although on those dates when lead was most toxic (November and April, Table 6), toxicity was observed by a drop in growth. As indicated previously, the growth rate (g) is also equal to the biomass production rate under our experimental conditions. For cadmium, copper, and mercury the reduction in growth rate was least (highest g''/g') in early summer and largest in late fall and winter. The cause for this is unknown although it appears to be due to either a change in the soluble fraction of the water or a change in the copepods themselves, since toxicity does not correlate well with particulate matter in the water. Although changes in the relative abundance of *C. vervalis* and *C. bicuspidatus thomasi* are difficult to determine because of the small size of most individuals, cyclopoids always composed over 95% of the copepods present. The effect of variations in diet (e.g. phytoplankton species composition) on resistance to metal toxicity is not known. Toxicity also does not correlate with control growth rates, since these are approximately the same year round when measured at 15°C, and it does not correlate with field temperatures, which reach a maximum in July and August when g''/g' is already decreasing again (Fig. 3, Borgmann et al. 1979). Toxicity of lead differs considerably from the other three cations. Lead is much less toxic than cadmium, copper, or mercury, and occasionally affects mortality more than growth. Furthermore, its seasonal cycle differs slightly and is related to the ash-free dry weight of the seston (Fig. 5).

In this study, a toxic concentration called the SC20 was defined. Although the SC20 results in only a 20% drop in biomass production rate (or equivalent mortality, eq. 9), since a considerable amount of growth occurs during the incubation period (mean $g' = 0.258$, corresponding to a 37-fold increase in size), the SC20 results in a drop in final biomass to about half that of the control. Hence, the SC20 is a highly toxic concentration. Published studies on the chronic toxicity of metals to freshwater zooplankton are rare, usually use *Daphnia* as the test organism, and employ a variety of methods for determining toxicity, thereby making comparisons with our results difficult. Biesinger and Christensen (1972) studied the effect of a large number of metals on the chronic toxicity to *D. magna* in Lake Superior water. Table 10 shows their chronic 3-wk LC50s and concentrations at which the number of young produced by newborn *Daphnia* within 3 wk at 18°C was reduced to half that of the control (50% reproductive impairment). Biesinger and Christensen

TABLE 10. The 50% reproductive impairment and 3-wk chronic LC50 concentrations for *Daphnia magna* in Lake Superior water (from Biesinger and Christensen 1972) compared to copepod SC20 values in Lake Ontario (Burlington Canal) water. All concentrations are in $\mu g \cdot L^{-1}$.

	Chronic toxicity to *Daphnia magna*		
Metal	50% reproductive impairment	3-wk LC50	Copepod SC20 concn.
Cadmium	0.7	2.42	5.7 to >80
Mercury	6.7	13	20 to 55
Copper	35	44	<20 to 80
Lead	100	300	240 to >10 000
Arsenic (as arsenate)	1400	2850	1380 to 3780

do not state whether reproductive impairment is due to direct inhibition of reproduction or due to slowed growth resulting in a time delay before reproduction occurs. It is interesting to note that both their reproductive impairment and LC50 for arsenic fall within the SC20 range for our copepods, in spite of the species and water chemistry differences. This may be related to the lack of seasonal variations observed in the toxicity of arsenic. The SC20 values for copper also overlap the reproductive impairment and LC50 for *Daphnia*, although toxicity of cadmium, mercury, and lead appear to be higher in the study by Biesinger and Christensen, especially if their reproductive impairment values are examined. This difference is not necessarily due to a species difference since the *Daphnia* were tested in softer water (alkalinity 42 $mg \cdot L^{-1}$) than the copepods (alkalinity ~88 $mg \cdot L^{-1}$).

Marshall (1978) observed that the carrying capacity in laboratory populations of *D. galeata mendotae* was reduced significantly at 4 $\mu g \cdot L^{-1}$ and equal to half that of the control at 7.7 $\mu g \cdot L^{-1}$. These concentrations are similar to the lowest copepod SC20 observed for this metal (5.7 $\mu g \cdot L^{-1}$). Winner and Farrell (1976) studied the effect of copper on four species of *Daphnia* and observed that the instantaneous rate of population increase was significantly reduced between 60 and 80 $\mu g \cdot L^{-1}$ in *D. magna* and between 40 and 60 $\mu g \cdot L^{-1}$ in the other three species. This again falls within the SC20 range for copepods (Table 10), although the toxicity to *D. magna* appeared to be lower than observed by Biesinger and Christensen (1972), possibly since Winner and Farrell used pond water of 100–119 $mg \cdot L^{-1}$ alkalinity.

It appears, therefore, that chronic toxicity of metals to natural copepod populations is similar to chronic toxicity to laboratory populations of *Daphnia*, especially for copper and arsenic, although cadmium, lead, and to a lesser extent mercury, may on occasion be considerably less toxic to copepods because of variations in natural conditions (e.g. seston effects on lead

TABLE 11. 48-h LC50 concentrations for three species of freshwater zooplankton (from Baudouin and Scoppa 1974).

Metal	48-h LC50 ($\mu g \cdot L^{-1}$) for:		
	Daphnia hyalina	*Eudiaptomus padanus padanus*	*Cyclops abyssorum prealpinus*
Cadmium	55	550	3800
Mercury	5.5	850	2200
Copper	5	500	2500
Lead	600	4000	5500

toxicity). This similarity in chronic toxicity, especially for copper, is particularly striking when compared to the large differences in acute toxicity of metals to copepods and daphnids. For example, some acute 48-h LC50s for cadmium, mercury, copper, and lead using adult copepods and *Daphnia* are summarized in Table 11 (from Baudouin and Scoppa 1974). For each metal, acute toxicity is highest for *Daphnia*, intermediate for the calanoid copepod, and lowest for the cyclopoid copepod. Since the copepods in the Burlington Canal were largely cyclopoids, it is evident that acute toxicity bears little relationship to chronic toxicity when comparing one type of zooplankter to another.

In the above discussion, the SC20 has been used to describe toxicity and to compare metal sensitivity of copepods to that of daphnids. However, unlike many measures of toxicity (e.g. LC50s), the SC20 has a further usefulness since its component parts, the G20 (concentration causing a 20% drop in biomass production rates) and the M20 (concentration causing a metal-induced mortality rate equal to 20% of the control production rate) can be used directly in equations (eq. 12, 13, and 14) to estimate the effect of metals on the dynamics of natural populations of copepods. To do this, the exponents c and b (listed in Tables 4, 6, and 8) must also be known. Since growth is usually affected at lower concentrations than mortality, the estimates of c and G20 (eq. 12 and 14) are particularly valuable. Fortunately, c appears to be relatively constant for any one metal. However, G20 varies seasonally for most metals studied (not for arsenic). Hence, an accurate model of the potential effect of metals on natural copepod populations requires either direct measurement of G20 on the date for which the model is desired, or further research on factors affecting G20 to permit its accurate estimation.

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ALLEN, K. R. 1971. Relation between production and biomass. J. Fish. Res. Board Can. 28: 1573–1581.

ANON. 1974. Analytical methods manual. Inland Waters Directorate, Water Quality Branch, Ottawa, Ont.

BAUDOUIN, M. F., AND P. SCOPPA. 1974. Acute toxicity of various metals to freshwater zooplankton. Bull. Environ. Control. Toxicol. 12: 745–751.

BIESINGER, K. E., AND G. M. CHRISTENSEN. 1972. Effects of various metals on survival, growth, reproduction, and metabolism of *Daphnia magna*. J. Fish. Res. Board Can. 29: 1691–1700.

BORGMANN, U. 1974. A theoretical description of biological rates. J. Theor. Biol. 45: 171–182.

BORGMANN, U., C. LOVERIDGE, AND R. COVE. 1979. A rapid method for the estimation of, and some factors affecting, copepod production rates in the Burlington Canal. J. Fish. Res. Board Can. 36: 1256–1264.

BORGMANN, O., O. KRAMAR, AND C. LOVERIDGE. 1978. Rates of mortality, growth, and biomass production of *Lymnaea palustris* during chronic exposure to lead. J. Fish. Res. Board Can. 35: 1109–1115.

EDMONDSON, W. T., AND G. G. WINBERG [ed.]. 1971. A manual on methods for the assessment of secondary productivity in fresh waters. IBP Handbook No. 17. Blackwell, Oxford. 358 p.

GOULDEN, P. D., AND P. BROOKSBANK. 1974. Automated atomic absorption determination of arsenic, antimony, and selenium in natural waters. Anal. Chem. 46: 1431–1436.

MARSHALL, J. S. 1978. Population dynamics of *Daphnia galeata mendotae* as modified by chronic cadmium stress. J. Fish. Res. Board Can. 35: 461–469.

WINNER, R. W., AND M. P. FARRELL. 1976. Acute and chronic toxicity of copper to four species of *Daphnia*. J. Fish. Res. Board Can. 33: 1685–1691.

WONG, P. T. S., Y. K. CHAU, AND P. L. LUXON. 1978. Toxicity of a mixture of metals on freshwater algae. J. Fish. Res. Board Can. 35: 479–481.

Effect of Number of Marked Fish and Years of Repetition on Precision in Studies of Contribution to a Fishery

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Methods are developed for predicting the expected precision for studies of the contribution of fish to a fishery, based upon the number of fish marked and the number of years an experiment is repeated. Studies concerned with estimating catch–release ratios, comparing catch–release ratios, and comparing distributions of catch are considered. It is suggested that releases of marked fish should be repeated for at least three or four broods, and often there is little advantage in releasing more than 50 000 marked fish per release group. Although we explicitly address studies of contribution to ocean fisheries, the methods apply directly to a broad range of studies involving marked fish, from evaluations of harvest rates on catchable-trout plants to estimates of catch–escapement ratios for Pacific salmon.

Key words: precision, experimental design, number of fish to mark, number of years to release marked fish, catch–release ratios, distribution of catch

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Des méthodes permettant de prédire la précision à laquelle on peut s'attendre dans les études de contribution des poissons à une pêcherie sont mises au point, fondées sur le nombre des sujets marqués et le nombre d'années de répétition de l'expérience. Nous traitons d'études ayant pour objet l'estimation des rapports captures–remises en liberté, la comparaison de ces rapports et enfin la comparaison des répartitions de captures. La remise en liberté de poissons marqués devrait être répétée avec au moins trois ou quatre portées, et très souvent, la remise en liberté de plus de 50 000 poissons marqués par groupe libéré n'offre que peu d'avantage. Bien que nous considérions explicitement des études de contribution en mer, les méthodes touchent directement une gamme étendue d'études comportant poissons marqués, depuis des évaluations de taux de récolte à même des peuplements de truites de taille pêchable jusqu'à des estimations de rapports capture–évasion chez les saumons du Pacifique.

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WITHIN the next 8 yr 150–200 million dollars are scheduled to be spent on new artificial propagation facilities for salmonids along the west coast of Canada and the United States. Large sums of money will be spent in estimating how many fish from these facilities are harvested, and where they are caught. It is important that these evaluation studies be designed to accomplish study objectives with minimum expense. It is therefore necessary to examine the relation between the number of years an experiment is repeated and the number of marked fish released each year, and the desired level of precision. In this paper we develop this relation and give examples for some typical situations.

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Precision

The primary objectives in different studies of contributions of fish to fisheries vary. In some studies the catch–release ratio (r) to specific fisheries is estimated by comparing the number of marked fish released with the number of marked fish harvested by various fisheries. Precision for these estimates refers to the width of the confidence interval about the estimate. Low values for precision mean narrow confidence intervals. Precision for normally distributed data is defined as one-half of the 100 $(1-\alpha)\%$ confidence interval, and is expressed as a proportion of r.

Some studies are designed to detect differences between the catch–release ratios for different groups of fish. Precision for these studies refers to the smallest difference between groups that is likely to be detected. Precision for normally distributed data is defined as the smallest difference—expressed as a pro-

portion of r—that can be detected with a $100\beta\%$ chance of concluding that two groups are not different when they do indeed differ (Type II error), and a $100\alpha\%$ chance of concluding that the groups are different when they are not (Type I error).

Other studies are concerned with the distribution of catch for different groups of fish. These studies are designed to detect differences between groups of fish in their relative contribution (p) to specific areas—the proportion of the total ocean catch of each group taken in specific areas. Precision again refers to the smallest difference between groups that is likely to be detected. Precision is expressed as a proportion of p and is defined as in the previous paragraph.

Assumptions

The present analysis includes several assumptions: the ocean catch of salmon is randomly sampled for marks, sampling intensity is constant geographically and temporally, there is no loss of marks, all marked fish examined are recognized, marked fish have the same survival and behavior as do unmarked fish, and the catch–release ratios for different broods are independent of each other.

The number of assumptions may appear excessive unless one remembers that the planning of studies is concerned here, rather than the analysis of data. In planning any study, the researcher must identify each assumption and make adjustments for those that are unacceptable. Studies should be planned to test the important assumptions, and the researchers must account for violations of those assumptions when analyzing the data.

Many of the formulae presented below are based on the additional assumption that r and p are normally distributed random variables. Since this is generally not true, the estimates of precision developed herein are actually indices of precision, which are ample for planning purposes. When analyzing data from a contribution study, one can usually overcome this problem by transforming the data (e.g. arc sine square root or log transformation; Snedecor and Cochran 1967).

Between-Brood and Within-Brood Variation

Variation about a mean value of r or p for releases of fish from several brood years is primarily a result of environmental and genetic fluctuations affecting broods differently, and is termed between-brood variation (B). Variation about an estimate of r or p for any one brood year is caused by sampling uncertainty, and is termed within-brood variation (W).

B is expressed as the standard deviation of the r or p values for the different broods being evaluated, divided by the mean of the estimate of r or p, respectively. W is expressed as the standard deviation of the estimate for r or p for one brood, divided by the estimate of r or p, respectively.

The number of marked fish released each year (M_o) directly affects W. We first develop the case for catch–release ratios, r, also termed contributions. Ignoring the fact that some species of fish are available to the ocean fisheries during more than 1 yr of their life, the expected number of mark recoveries from a release group, $E(m)$, is

$$E(m) = SrM_o$$

where S is the proportion of the total catch sampled for marks. Solving for r and substituting m for its expected value, an estimate of r is

$$r = \frac{m}{SM_o} = \frac{N}{\nu}\frac{m}{M_o},$$

where N is the total catch of marked and unmarked fish and ν is the number of fish examined for marks ($S = \nu/N$). Since the catch N is itself usually an estimate, the variance of r is

$$V(r) = \left(\frac{1}{M_o}\right)^2\left(\frac{1}{\nu}\right)^2[N^2V(m) + m^2V(N) + V(m)V(N)]$$

where $V(m)$ and $V(N)$ are the variances of m and N, and the finite population correction is omitted. As shown by Goodman (1960), an unbiased estimate of $V(r)$ is

$$v(r) = \left(\frac{1}{M_o}\right)^2\left(\frac{1}{\nu}\right)^2[N^2v(m) + m^2v(N) - v(m)v(N)]$$

where $v(N)$ is an unbiased estimate of $V(N)$ and $v(m)$ is an unbiased estimate of $V(m)$.

Our analysis of data (California Department of Fish and Game, unpublished) indicates that the ocean catch N is estimated within 10% of the true value. So,

$$2[v(N)]^{1/2} = 0.1\,N$$

and

$$v(N) = 0.0025\,N^2.$$

Since the number of marked fish observed, m, is generally a very small proportion of the number of marked fish released, we have assumed that m follows the poisson distribution so that, $v(m) = m$. Substituting for m, $v(N)$, and $v(m)$,

$$v(r) = r^2\left(0.0025 + \frac{0.9975}{SrM_o}\right)$$

and

$$(1)\qquad W(r) = \left[\frac{v(r)}{r^2}\right]^{1/2} = \left[0.0025 + \frac{0.9975}{SrM_o}\right]^{1/2}.$$

For many situations an approximation is adequate,

$$(1a)\qquad W(r) \cong [SrM_o]^{-1/2}.$$

The comparable development for distribution of catch requires consideration of the proportion p of the total ocean harvest of a group of marked fish taken in a certain area. By definition,

$$p = m/m_T,$$

TABLE 1. Between-brood variations for catch-release ratios $B(r)$ calculated from available data. The catch–release ratios are given in parentheses.

Species	Hatchery	Catch area			
		Coast-wide	Oregon	Washington	British Columbia
Coho salmon	Cowlitz[a]	0.37 (0.033)	0.35 (0.008)	0.39 (0.025)	—
Fall chinook salmon	Spring Creek[b]	0.70 (0.0029)	1.16 (0.0002)	0.72 (0.0016)	0.63 (0.0010)
	Kalama Falls[b]	0.38 (0.0024)	0.43 (0.0001)	0.50 (0.0008)	0.43 (0.0015)
	Combined Columbia River hatcheries[b]	0.39 (0.0022)	0.70 (0.0001)	0.37 (0.0011)	0.40 (0.0010)
	Coleman[c]	0.33 (0.0059)	—	—	—
	Nimbus[c]	0.71 (0.0044)	—	—	—

[a]Releases of 1967–69 broods (Washington Department of Fisheries, unpublished manuscript 1976). Cowlitz hatchery is on the Columbia River system.

[b]Releases of 1961–64 broods (Worlund et al. 1969; Rose and Arp 1970; Arp et al. 1970; Wahle et al. 1972). These hatcheries are on the Columbia River system.

[c]Releases of 1968–70 broods (R. J. Hallock and R. R. Reisenbichler unpublished data). These hatcheries are on the Sacramento River system, CA.

where m is the number of mark recoveries in the area of interest and m_T is the number of mark recoveries from the total ocean harvest. If r_T is the total contribution of this group of fish to all the ocean fisheries, then

$$m_T = r_T S M_o$$

and

$$p = \frac{m}{r_T S M_o}.$$

Employing the same argument used in developing eq. (1),

$$v(p) = \left(\frac{1}{r_T}\right)^2\left(\frac{1}{\nu}\right)^2\left(\frac{1}{M_o}\right)^2[N^2 v(m) + m^2 v(N) - v(m)v(N)]$$

and

$$(2) \quad W(p) = \left[\frac{v(p)}{p^2}\right]^{1/2} = \left[0.0025 + \frac{0.9975}{pSr_T M_o}\right]^{1/2}.$$

Some studies will be designed with several replicates within each experimental group of fish each year; however, the W of interest is a property of the total release for each experimental group, not of each replicate. Consequently, M_o is defined as the number of marked fish released per experimental group per year, not the number per replicate.

Estimating Catch–Release Ratios

Precision K for estimating catch–release ratios is related to B and the number of years an experiment is repeated, n, as follows:

$$(3) \quad K = (t_\alpha)B/n^{1/2}.$$

The t_α has $(n - 1)$ degrees of freedom.

Within-brood variation affects the observed between-brood variation. If B_o is the between-brood variation when $W = 0$,

$$(4) \quad B = [W^2 + B_o^2]^{1/2}.$$

Now precision K can be predicted for different values of M_o when r, B_o, and n are known. First, eq. (1) is used to calculate W for each M_o of interest. Then eq. (4) is used to calculate B for each value of W, and eq. (3) to calculate K for the values of n and B. Once reasonable values for r and B_o are chosen and the relation between K, and M_o and n is determined, specific values for M_o and n can be identified that will give the desired level of precision.

EXAMPLE 1

If the expected catch–release ratio, r, is 0.01, a value of 0.40 for B_o seems reasonable (Table 1). For this example the intensity of mark sampling is 20% ($S = 0.20$), the 90% confidence interval is to be considered, and 5000 marked fish are released each year. The determination of the expected precision K for 3 yr of releases begins by substituting into eq. (1),

$$W = \left[0.0025 + \frac{0.9975}{(0.2)(0.01)(5000)}\right]^{1/2} = 0.32.$$

From eq. (4)

$$B = [0.32^2 + 0.40^2]^{1/2} = 0.51,$$

and from eq. (3)

$$K = (2.92)(0.51)/[3]^{1/2} = 0.86,$$

where 2.92 is the two-tailed value of t for $\alpha = 0.10$ and two degrees of freedom. Repetition of the above procedure for different values of M_o and n produces the data shown in Fig. 1.

Comparing Catch–Release Ratios

This section addresses experimental designs where catch–release ratios (r values) for two or more groups of fish are compared from one catch area for several years. For example, one may wish to compare the total ocean

contributions of different stocks or different sized smolts reared at the same hatchery. It is likely that the r values from the different groups of fish being compared are correlated, because all the groups will do better in years when ocean conditions are good than in years when they are poor. For the case when two groups are to be compared, $d_i = r_{a_i} - r_{b_i}$, where r_{a_i} and r_{b_i} are the contributions from the i^{th} brood year of groups a and b, respectively. The variance of d_i is

$$V(d_i) = (\sigma_{r_a})^2 + (\sigma_{r_b})^2 - 2R\sigma_{r_a}\cdot\sigma_{r_b}$$

where $\sigma^2_{r_a}$ is the variance of the r values for group a, $\sigma^2_{r_b}$ is the variance of the r values for group b, and R is the coefficient of correlation between the r values for group a and group b. If $\sigma^2_{r_a} = \sigma^2_{r_b} = \sigma^2$, the variance of d_i is estimated by

$$v(d_i) = 2s^2(1 - R),$$

where s is the estimate for σ. Precision relative to $\bar{r}_a$ is

$$\Delta = (t_\alpha + t_\beta)B\left[\frac{2(1 - R)}{n}\right]^{1/2}, \tag{5}$$

where $B = s/\bar{r}_a$. The two-tailed t value is used for t_α and the one-tailed t value is used for t_β (Cochran and Cox 1957). The degrees of freedom (df) for the t values come from the generalized analysis of variance table:

Source	df
Group	$v - 1$
Year	$n - 1$
Group × Year	$(v - 1)(n - 1)$
Error	$vn(z - 1)$

Here v is the number of groups of fish to be compared, n is the number of years marked fish are released, and z is the number of replicates per group each year. Because this is a mixed model (the Group effect is a fixed effect and the Year effect is a random effect) the degrees of freedom for the interaction term $(v - 1)(n - 1)$ are used to determine the t values. Clearly, the interaction term cannot be tested for significance when there is no replication.

Three studies provide data which allow us to calculate correlation coefficients. Data from a study in Washington comparing return rates for three broods of late and early running stocks of coho salmon, *Oncorhynchus kisutch* (Washington Department of Fisheries, unpublished manuscript 1976), were used to calculate correlation coefficients of 0.76, 0.97, and 0.93 for contributions to Oregon, Washington, and the combined ocean fisheries, respectively. Data from a study comparing four broods of fall chinook salmon, *O. tshawytscha*, from two Columbia River hatcheries (Worlund et al. 1969; Rose and Arp 1970; Arp et al. 1970; Wahle et al. 1972) were used to calculate correlation coefficients of 0.22, 0.59, and 0.55 for contributions to Washington, British Columbia, and the combined ocean fisheries, respectively. Data from a study in California comparing releases at the hatcheries and into the Sacramento – San Joaquin estuary for three broods of American River and six broods of upper Sacramento River fall chinook salmon (R. J. Hallock and R. R. Reisenbichler, California Department of Fish and Game, Anadromous Fisheries Branch, CA, 1979, unpublished data) were used to calculate correlation coefficients of 0.54 and 0.95, respectively, for contributions to the combined ocean fisheries.

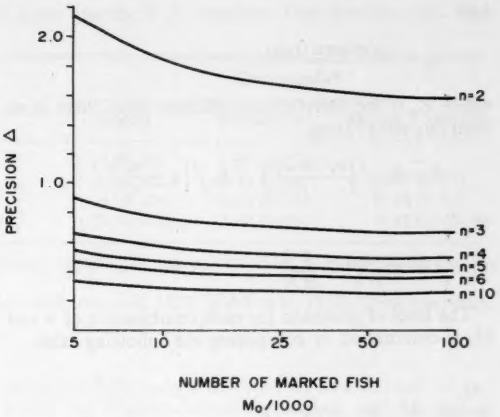

FIG. 1. Effect of number of marked fish released each year, M_o, and the number of years releases are repeated, n, on the expected precision for estimating catch–release ratios K when $r = 0.010$, $S = 0.20$, $B_o = 0.40$, and $\alpha = 0.10$.

The correlation between the r values for different groups of fish decreases as the precision for estimates of the individual r values deteriorates, i.e. as within-brood variation W increases. For contributions r_a and r_b from two groups of fish, the observed value of each r is the sum of the true contribution γ and the error due to sampling ϵ:

$$r_{a_i} = \gamma_{a_i} + \epsilon_{a_i}$$

and

$$r_{b_i} = \gamma_{b_i} + \epsilon_{b_i}.$$

Consequently, the variances are

$$V(r_a) = V(\gamma_a) + V(\epsilon_a)$$

and

$$V(r_b) = V(\gamma_b) + V(\epsilon_b).$$

The equation for the correlation coefficient is

$$R = \frac{\text{covariance } (r_a r_b)}{[V(r_a)V(r_b)]^{1/2}}.$$

If $V(\gamma_a) = V(\gamma_b) = \sigma_\gamma{}^2$, and $V(\epsilon_a) = V(\epsilon_b) = \sigma_\epsilon{}^2$, we have

$$R = \frac{\text{covariance } (r_a r_b)}{\sigma_\gamma{}^2 + \sigma_\epsilon{}^2}$$

and

$$R_o = \frac{\text{covariance } (r_a r_b)}{{\sigma_\gamma}^2},$$

where R_o is the correlation coefficient when there is no sampling error. Thus,

$$R = R_o \Big/ \left(\frac{{\sigma_\gamma}^2 + {\sigma_\epsilon}^2}{{\sigma_\gamma}^2}\right) = R_o \Big/ \left(1 + \frac{{\sigma_\epsilon}^2}{{\sigma_\gamma}^2}\right)$$

or

$$R = R_o \Big/ \left(1 + \frac{W^2}{{B_o}^2}\right). \quad (6)$$

The level of precision for each combination of n and M_o is determined by completing the following table.

M_o (given)	W	B	W/B_o	R	n 2	3	4	. . .
5 000	W_1	B_1	W_1/B_o	R_1	Δ_{12}	Δ_{13}	Δ_{14}	. . .
10 000	W_2	B_2	W_2/B_o	R_2	Δ_{22}	Δ_{23}	Δ_{24}	. . .
.								
.								
.								

W is computed from eq. (1), B is computed from eq. (4) after one decides upon an expected B_o (Table 1), and R is computed from eq. (6) after one chooses an expected R_o. Precision Δ is calculated according to eq. (5). Clearly, M_o has a greater effect on precision when there is correlation than when there is no correlation (Fig. 2 and 3).

EXAMPLE 2

If the expected catch–release ratio, r, of the control group is 0.001, a value of 0.40 for B_o seems reasonable (Table 1). For this example, $S = 0.20$, $\alpha = 0.10$, $\beta = 0.20$, $R_o = 0.65$, and $M_o = 25\,000$. From eq. (1), $W = 0.45$, and from eq. (4), $B = 0.60$. Using eq. (6) and $W/B_o = 0.45/0.40 = 1.1$, $R = 0.29$. For 3 yr of repetition ($n = 3$) there are two degrees of freedom, so $t_{10\%} = 2.92$ (two-tailed value) and $t_{20\%} = 1.06$ (one-tailed value). From eq. (5)

$$\Delta = (2.92 + 1.06)(0.6)\left[\frac{2(1 - 0.29)}{3}\right]^{1/2} = 1.64,$$

which corresponds to the data shown in Fig. 3.

Comparing Distribution of Catch

In studies in which distributions of catch are compared, the expected precision is calculated separately for each catch area of interest. The same calculations are performed to estimate precision for comparing relative contributions to one catch area (p) as were used for comparing catch–release ratios, except that eq. (2) is used to calculate W instead of eq. (1). Table 2 presents some observed between-brood variations for relative ocean contributions. The relations between expected precision for distribution of catch and numbers of marked fish and years of repetition are similar to those for estimating and comparing contributions (Fig. 4).

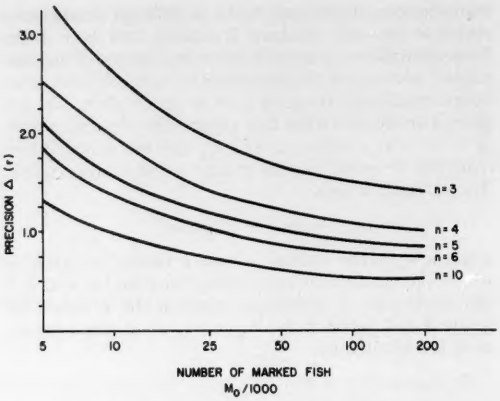

FIG. 2. Effect of number of marked fish released per year in each group, M_o, and the number of years releases are repeated, n, on the expected precision for comparing catch-release ratios, Δ, when $r = 0.001$, $S = 0.20$, $B_o = 0.40$, $\alpha = 0.10$, and $\beta = 0.20$.

No evidence was found that relative ocean contributions are positively correlated in group comparisons. If there is reason to expect correlation, the steps outlined in the previous section for using R in calculating precision also apply to distribution of catch studies.

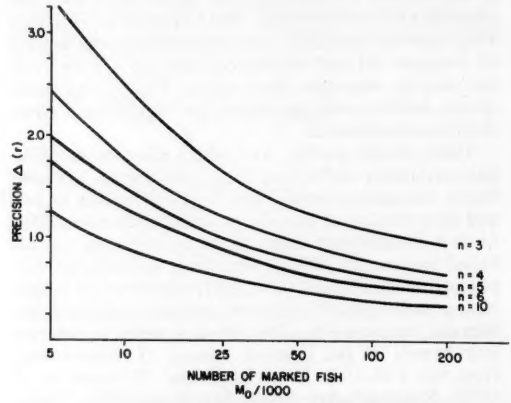

FIG. 3. Effect of number of marked fish released per year in each group, M_o, and the number of years releases are repeated, n, on the expected precision for comparing catch–release ratios, Δ, when $R_o = 0.65$, $r = 0.001$, $S = 0.20$, $B_o = 0.40$, $\alpha = 0.10$, and $\beta = 0.20$.

TABLE 2. Between-brood variations for relative contributions to ocean fisheries, $B(p)$, calculated from available data. The relative contributions to each fishery are given in parentheses.

Species	Hatchery	Catch area		
		Oregon	Washington	British Columbia
Coho salmon	Cowlitz[a]	0.19 (0.24)	0.07 (0.75)	—
Fall chinook salmon	Spring Creek[b]	0.65 (0.053)	0.11 (0.60)	0.19 (0.35)
	Kalama Falls[b]	0.35 (0.028)	0.30 (0.34)	0.18 (0.62)
	Combined Columbia River hatcheries[b]	0.70 (0.055)	0.08 (0.49)	0.17 (0.45)

[a]Releases of 1967–69 broods (Washington Department of Fisheries, unpublished manuscript 1976). Cowlitz hatchery is on the Columbia River system.

[b]Releases of 1961–64 broods (Worlund et al. 1969; Rose and Arp 1970; Arp et al. 1970; Wahle et al. 1972). These hatcheries are on the Columbia River system.

Discussion

Six basic equations are presented for use in calculating expected precisions. The figures were developed by using these six equations. Once a graph representing the situation at hand has been developed, the appropriate number of marked fish and number of years an experiment is to be repeated can be determined for a desired level of precision.

Investigators must define the geographic area(s) of interest, obtain appropriate estimates of r, or p and r_T, and B_o, determine the feasibility of replication, and define the desired level of precision. For comparison studies they must also determine the number of groups to be compared and a realistic value for R_o. Inferences about appropriate values for B_o and R_o must often be based on limited data. The best tactic is to choose optimistic and pessimistic values for both B_o and R_o, and to examine the relation between precision, and M_o and n for both sets of values. A conservative approach is to choose the number of years of repetition from the pessimistic analysis and the number of marked fish per group from the optimistic analysis. If the pessimistic situation occurs, one has repeated the experiment enough years to achieve satisfactory precision. If the optimistic situation occurs, one has released enough marked fish to realize the superior precisions. When one is interested in more than a single catch area, the relationship between precision, and n and M_o for each area is examined separately before values for n and M_o are selected. When one is interested in estimating contributions during portions of the fishing season, one simply uses values for p, r, and B_o that are appropriate for the time intervals under consideration.

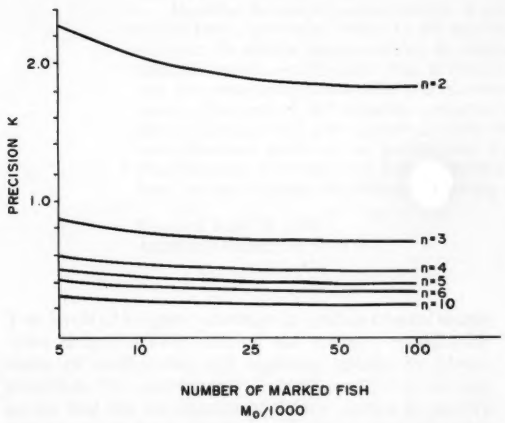

FIG. 4. Effect of number of marked fish released per year in each group, M_o, and the number of years releases are repeated, n, on the expected precision for comparing relative contributions, Δ, when $r_T = 0.10$, $S = 0.20$, $p = 0.20$, $B_o = 0.20$, $\alpha = 0.10$, $\beta = 0.20$.

Although this paper specifically addresses only ocean contribution studies, it applies to a broad range of studies involving marked fish, from evaluations of harvest rates on catchable-trout plants to estimates of catch–escapement ratios for Pacific salmon. Precisions for such studies can be estimated by substituting appropriate values for r, S, B_o, and R_o, and, when considering studies not designed to estimate the contributions of Pacific salmon to ocean fisheries, using eq. (1a) or a modification of eq. (1). This paper also provides the tools to determine the most efficient sampling intensities by examining the effects of different sampling intensities on the precision and cost of contribution studies.

The figures demonstrate the importance of repeating experiments for more years than has been the custom in the past. Researchers often concentrate on reducing within-brood variation and only address between-brood variation superficially in their planning. The resultant studies yield imprecise estimates of contribution which could have been obtained with less expense by marking fewer fish. Our experience suggests that releases of marked fish should be repeated a minimum of three or four broods for contribution studies, and often there is little advantage in releasing more than

50 000 marked fish per release group. Of course, studies in which a large correlation coefficient is expected and studies designed to detect relationships between contribution and environmental variables are exceptions. The latter require good precision for the contribution of each brood, since relationships can be masked when values are measured inaccurately. Such studies must extend for more years and require more marked fish per brood than studies designed simply to estimate or compare contributions. As more studies are repeated for longer periods of time, better estimates of B_0 and R_0 will be obtained. These improved estimates will facilitate more efficient design of future studies.

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ARP, A. H., J. H. ROSE, AND S. K. OLHAUSEN. 1970. Contribution of Columbia River hatcheries to harvest of 1963 brood fall chinook salmon (*Oncorhynchus tshawytscha*). U.S. National Marine Fisheries Service, Columbia Fisheries Program Office, Portland, Oregon. 33 p.

COCHRAN, W. G., AND G. M. COX. 1957. Experimental designs. 2nd ed. Wiley, New York, N.Y. 611 p.

GOODMAN, L. A. 1960. On the exact variance of products. J. Am. Stat. Assoc. 42: 708–713.

ROSE, J. H., AND A. H. ARP. 1970. Contribution of Columbia River hatcheries to harvest of 1962 brood fall chinook salmon (*Oncorhynchus tshawytscha*). U.S. Bur. Comm. Fish., Columbia Fisheries Program Office, Portland, Oreg. 27 p.

WAHLE, R. J., A. H. ARP, AND S. K. OLHAUSEN. 1972. Contribution of Columbia River hatcheries to harvest of 1964 brood fall chinook salmon (*Oncorhynchus tshawytscha*). U.S. National Marine Fisheries Service, Columbia Fisheries Program Office, Portland, Oreg. 31 p.

WORLUND, D. D., R. J. WAHLE, AND P. D. ZIMMER. 1969. Contribution of Columbia River hatcheries to harvest of 1961 brood fall chinook salmon (*Oncorhynchus tshawytscha*). U.S. Fish. Wildl. Serv. Fish. Bull. 67: 361–391.

Assessment of the Importance of Nutrient Recycling by Seabirds in the St. Lawrence Estuary

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Feeding and excretion rates of the herring, great black-backed, and ring-billed gulls (*Larus argentatus*, *L. marinus*, and *L. delawarensis*), and of the common eider (*Somateria mollissima*) were measured in captive individuals and the concentration of soluble nutrients in their excreta was established.

The bird population in a 30.6-km² coastal study area varied between 2500 and 12 500 individuals between early May and mid-November. These birds excreted a seasonal total of 5.8, 4.2, and 48.1 kg-at. of soluble silicate, phosphate, and nitrogenous compounds, respectively. Such quantities, when introduced in the nutrient budget of the coastal area studied, are found to be negligible, considering the levels of nutrients generally recorded in these waters on the one hand, and the relative importance of alternate sources such as land drainage and vertical mixing on the other. Thus, the seabirds can hardly be viewed, except perhaps under the most special circumstances, as important agents in the dynamic nutrient regeneration processes of marine coastal waters of the St. Lawrence Estuary.

Key words: nutrient recycling, seabirds, St. Lawrence Estuary

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Nous avons mesuré les taux d'ingestion et d'égestion ainsi que la concentration en éléments minéraux solubles dans les excréments d'oiseaux aquatiques captifs appartenant aux espèces suivantes : le goéland argenté, le goéland à manteau noir et le goéland à bec cerclé (*Larus argentatus*, *L. marinus* et *L. delawarensis*) et l'eider à duvet (*Somateria mollissima*).

Du début de mai à la mi-novembre, la population d'oiseaux occupant notre aire d'étude de 30.6 km² a varié entre 2500 et 12 500 individus et y a excrété une masse totale de 48.1, 5.8 et 4.2 kg-at. de dérivés azotés solubles, de silicates et de phosphates, respectivement. Pareilles quantités semblent négligeables dans le bilan des sels nutritifs du milieu côtier étudié, compte tenu des concentrations naturelles généralement élevées de ces substances dans les eaux estuariennes d'une part et de l'importance relative très considérable d'autres sources de minéraux, dont le mélange vertical et l'apport de cours d'eau, d'autre part. A la lumière de ces résultats, nous concluons qu'il n'est pas justifié, si ce n'est peut-être dans des circonstances tout à fait exceptionelles, d'attribuer aux communautés d'oiseaux aquatiques un rôle important dans le bilan des sels nutritifs pour les eaux littorales de l'estuaire du Saint-Laurent.

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THE levels of biogenic elements in surface coastal waters often drop markedly during the summer months because of continuous and vigorous uptake by phytoplankton. Not surprisingly, several workers have suggested that the enrichment of littoral waters by seabird excreta could have important effects in increasing primary and secondary productivity, not only in the immediate vicinity of nesting seabird colonies, but in wider coastal areas as well (Uspenski 1959; Tuck 1961; Golovkin 1963; Golovkin and Zelickman 1965; Golovkin and Pozdnyakova 1966; Golovkin et al. 1972; Zelickman and Golovkin 1972; and others). Experiments in the laboratory have shown that seabird droppings added to seawater could induce significant increases in phytoplankton cell numbers (Golovkin 1967). Galkina (1974) further showed that 17% (dry wt) of the excreta of fish-eating seabirds were water-soluble: of this, 17% consisted of mineral salts while the remainder was made up of soluble organic

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compounds. The latter were also shown to stimulate the photosynthetic activity of the phytoplankton in the laboratory (Galkina 1977).

A number of Russian workers have suggested that seabirds played a considerable role in nutrient budgets for coastal waters (Golovkin 1976; Golovkin and Garkavaya 1975; Golovkin and Gurevich 1974; Golovkin et al. 1972; Golovkin and Zelickman 1965; Zelickman and Golovkin 1972). We feel, however, that their conclusions were based upon rather limited observation series and that their choice of control observation sites was questionable. In this paper, we present direct measurements of the excretion rates of various species of captive aquatic birds, empirical determinations of the concentration of soluble nutrients in their excreta, and an assessment of the importance of these animals in recycling nutrients along a typical coastal area of the St. Lawrence Estuary.

Materials and Methods

Excreta were obtained from nearly full-grown hand-reared juveniles of the following birds: two herring gulls (*Larus argentatus*), two great black-backed gulls (*L. marinus*), three ring-billed gulls (*L. delawarensis*), and five common eiders (*Somateria mollissima*). The birds were housed in four screen-floored 1-m² roofed outdoor cages, equipped with a drawer lined with several layers of polyethylene. Prior to the experiment, the birds were given a 24-h preliminary training period during which they were caged and fed as in the experimental program, but no excreta were collected. The gulls were fed (ad libitum) fresh frozen herring (*Clupea harengus*) and the eiders fresh frozen small blue mussels (*Mytilus edulis*). Detailed records of both food consumption and excretion (dry wt) were made for the duration of the 72-h experiment. Excreta from each cage were recovered twice a day (0830 and 1200) by scraping and washing the top polyethylene sheet and adjusting the sample volume to 1 L with distilled water. Half of the homogenized solution was frozen for dry weight determination. The remainder was further divided into five, 100-mL subsamples (five replicates) and placed in 4.7-L plastic jerry cans filled with filtered (GF/C glass fiber) seawater collected at Métis Beach (Fig. 1). A sixth jerry can containing seawater only served as a control. All jerry cans were stoppered and attached to a floating device that allowed their regular sampling and incubation in nearshore surface waters at Métis Beach for 48 h. The incubation temperature at the site varied between 10 and 15°C and wave action ensured continuous stirring. Every 8 h, a 150-mL sample was drawn off each jerry can, filtered (GF/C glass fiber filter), decanted into three, 60 mL polyethylene bottles, and frozen. These subsamples served later for the determination of the levels of NH_3 NO_2^{2-}, NO_3^{3-}, SiO_3^{2-}, PO_4^{3-} following the standard methods described in Strickland and Parsons (1972) using a Technicon auto-analyser.

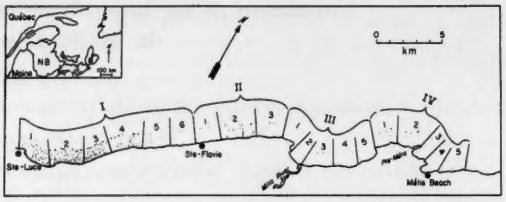

FIG. 1. Location of the study area and position of the bird sampling stations.

Representative samples of food and excreta were freeze-dried and calorific values of both established, using an adiabatic bomb calorimeter.

Seabird counts were made in calm weather at weekly intervals between Sainte-Luce and Métis Beach (Fig. 1) from May to November 1978. A spotting scope was used to cover an approximate maximum distance of 1 km offshore. The total area censused was 30.6 km².

Results and Discussion

The food consumption figures obtained are shown in Table 1 and compared to predicted values derived using the equation of Lasiewski and Dawson (1967) for non-passerine birds. This estimate of Basal Metabolic Rate (BMR), once multiplied by 3.5, represents a fair estimate of the total Daily Energy Expenditure (DEE) by a free-living bird (King 1974). Sizable differences are noted between predicted (PFC) and measured (MFC) values of food consumption (Table 1). The three ring-billed gulls, for instance, consumed food energy at a

TABLE 1. Measured (3-d average) and predicted (3.5 × predicted *BMR* calculated from Lasiewski and Dawson (1967)) daily food consumption, daily excrement production, and digestibility. Consumption and production are expressed in both dry weight (g) and calorific value (kJ). Eiders were fed blue mussels (1.26 kJ·g⁻¹) and gulls, herring (26.3 kJ·g⁻¹) ad libitum.

	Food consumption				Excrement production				
	Predicted		Measured		Predicted		Measured		Total digestibility (%)
	g	kJ	g	kJ	g	kJ	g	kJ	
Common eider (*Somateria mollissima*)	1615	2037	356	449	771	0	170	0	100
Herring gull (*Larus argentus*)	44	1234	50	1415	14	205	16	231	84
Great black-backed gull (*Larus marinus*)	59	1659	86	2436	19	291	28	433	82
Ring-billed gull (*Larus delawarensis*)	26	722	69	1948	10	172	26	475	76

[a]Average bird weight (kg) of the common eider taken as 2.2 (St. Lawrence Estuary adults), herring gull 1.1 (Threlfall and Jewer 1978), great black-backed gull 1.7 (Belopol'skii 1961), and ring-billed gull 0.52 (Ryder 1978).

TABLE 2. Amounts of soluble nutrients per gram (dry wt) of excreta and per bird per day (see Table 1).

Nutrient	Common eider (*Somateria mollissima*)		Herring gull (*Larus argentatus*)		Great black-backed gull (*Larus marinus*)		Ring-billed gull (*Larus delawarensis*)	
	Per gram of excreta (μg-at.)	Per bird (mg-at.)	Per gram of excreta (μg-at.)	Per bird (mg-at.)	Per gram of excreta (μg-at.)	Per bird (mg-at.)	Per gram of excreta (μg-at.)	Per bird (mg-at.)
NH_3	58.2	44.9	1048.0	14.7	1288.0	24.5	1579.0	15.8
NO_2^{2-}	0.5	0.4	1.5	0.1	2.8	0.1	5.5	0.1
NO_3^{3-}	5.9	4.5	34.3	0.5	50.8	1.0	70.0	0.7
N_{tot}[a]	64.7	49.9	1083.0	15.2	1341.0	25.5	1654.0	16.5
SiO_2^{2-}	8.8	6.8	34.3	0.5	12.7	0.2	11.3	0.1
PO_4^{3-}	3.5	2.7	348.0	4.9	479.0	9.1	550.0	5.6

[a] N_{tot} taken as the sum of NH_3, NO_2^{2-}, and NO_3^{3-} concentrations.

rate equivalent to nearly 10 times BMR: the birds were restless, not adjusting to the confined conditions of the holding pen, and were also completing some body and feather growth. The eiders reacted very differently, resting prone and eating little during the first half of the experiment. We therefore felt that the theoretical values of food consumption would constitute a more reliable basis for calculations and have done so throughout this article. We also assumed that nutrient composition and solubility of the excreta were not affected by these circumstances.

Measured values of Excrement Production (MEP) are also presented in Table 1. Predicted Excrement Production (PEP) therein was derived from the expression

$$\text{PEP} = \frac{\text{PFC}}{\text{MFC}} \times \text{MEP}.$$

Calculated values of 170 and 230 g (fresh wt; using a 12.1 fresh/dry ratio) of excreta/bird/day for the herring and the great black-backed gulls, respectively, are in agreement with values of 160 and 200 calculated for these same species by Golovkin and Garkavaya (1975).

As can be noted in Table 1, the mass of excreta produced by a single common eider during a day is 40 times greater than that produced by a larid, such as the great black-backed gull, of nearly the same body weight. This is easily explained by differences in the diet of the birds, the excreta of the eider being almost wholly mineral, and made up of finely ground mussel shells.

Captivity may induce morphological changes in birds (gizzard size, intestine length, etc.: see Owen 1975 for references) that could influence the metabolic functions of the animals and the characteristics of their excreta. However, the high values of digestibility (or assimilation efficiency) displayed by our captive birds (Table 1) indicate that the metabolic functions performed normally. So, we assume that the excreta produced by our captives differed neither physically nor chemically from those produced by free-living individuals.

The amounts of soluble nutrients per gram of excreta (dry wt) and per bird for each species are presented in Table 2. The concentrations of nutrients in the excreta of the three larids are of the same relative magnitude but much higher than that of the eider. Again, this difference is due to the peculiar diet of the latter. However, when comparisons are made in nutrient concentrations per bird, the discrepancy largely disappears, suggesting that neither phylogeny nor diet profoundly alter the chemical characteristics of the excreta of seabirds. When bird species are compared, the eiders seem more important soluble nitrogen and silicon producers and less important phosphate excretors than the gulls (Table 2). The concentrations of soluble phosphate detected in the excreta of the gulls and the eider during this study are of the same order of magnitude as the estimated values given by Golovkin et al. (1972) for the thick-billed murre (*Uria lomvia*) and for the kittiwake (*Rissa tridactyla*). Our average nitrogen value in the excreta of the four birds studied is 123 times smaller than that given by Steven (1974) for the gannet (*Sula bassana*) around Bonaventure Island in the Gulf of St. Lawrence. (Steven apparently based his calculations upon data published by Hutchinson (1950).) This discrepancy cannot be explained by phylogenetic or metabolic differences between the Sulidae on the one hand and the Laridae and Anatidae on the other.

The results of the bird censuses are presented in Fig. 2 and are expressed in birds/km^2. However, bird distribution was not random and the implications of this patchiness will be discussed later. Eider abundance peaks twice in the summer: the first occurs in June–July when juveniles and females that have failed in their breeding attempt make up the bulk of the population (beginning in early August, juveniles can no longer be safely distinguished from adults and are tallied with them); a second, larger peak occurs in September when flocks of molting, transient males drift through the

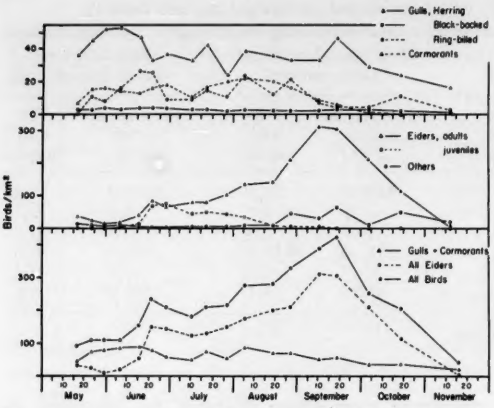

FIG. 2. Results of the bird censuses (in birds/km²) over the 30.6-km² coastal area shaded in Fig. 1, during the summer 1978. The cormorant is the double-crested *Phalacrocorax auritus* while the category "Other birds" includes mainly the black duck (*Anas rubripes*), garrows (*Bucephala* sp.), and scoters (*Melanitta* sp.). For the purposes of nutrient production estimates, these were equated to the gull species which they resembled most in size.

study area, heading eastward (Fig. 2). From mid-June to mid-October, about 5000 eiders were present in the 30.6 km² study area while over 12 000 birds were recorded at times. Gulls were far less abundant with their numbers varying between 1200 and 2400 individuals. The composition of the bird community in the stretch of shoreline studied here is quite representative of the situation encountered throughout the St. Lawrence Estuary (Gauthier and Bédard 1976; Reed 1975).

Using the census results and the rates of excrement production, we calculated the total quantity of excreta (dry wt) released in the study area during the entire summer. The eiders produced 634 t (1 t = 1000 kg), the total gull population 3.3 t, and all the other aquatic birds present (see legend of Fig. 2 for details) an estimated 13.2 t. The calculated daily total excrement production by all the birds in the study area varied from 0.9 t on May 17, to a high of 7.4 t on September 19 and 0.07 t on November 16, the day of the last census. The daily summer average stood at 3.7 t and the eiders accounted for 97.5% of this value.

Using the amounts of soluble nutrients per gram (dry wt) of excreta per bird per day given in Table 2 and the results of the bird censuses, we calculated the quantity of soluble nutrients released by the entire bird community on each census date over the whole study area. The results (Table 3) indicate that the entire summer production of soluble nitrogenous compounds (NO_2^{2-}, NO_3^{3-}, NH_3) readily available for photosynthesis stood at 48.1 kg-at. (of which 91% was released in the form of ammonia). Soluble silicate and phosphate quantities were 5.8 and 4.2 kg-at., respectively. The average daily nutrient release by the entire bird population varied between 4.4, 0.4, and 0.7 g-at./km² and 17.6, 2.3, and 1.2 g-at./km² of soluble nitrogen, silicate, and phosphate, respectively. Therefore, the seabirds were enriching the top 1-m layer of the study area at a daily rate (over the 183-d study) of 0.02 μg-at./L of soluble nitrogenous compounds of 0.002 μg-at./L of soluble silicate and of 0.001 μg-at./L of phosphate. In the calculations above, the dispersion of the excreta was considered uniform over the whole area. Quite obviously, such a method leads to estimating minute and insignificant amounts of nutrients. However, as mentioned above, the excreted matter is released in a rather narrow intertidal strip or near reefs or points where the birds tended to aggregate. For instance, one large flock of 3000 molting male eiders gathered near Pointe Métis (census area IV.5, Fig. 1) for about 1 wk (census period 15, Table 2). The raft covered roughly 0.3 km² and influenced a surface area of about 0.6 km² through daily shifts in position. The daily contribution of soluble nitrogen to the littoral waters by these birds was established at 0.25 μg-at. $\cdot L^{-1}$ (considering a 1-m-deep water column), of which 90% was ammonia, a soluble form of nitrogen known to be taken up preferentially by planktonic algae (Strickland et al. 1969). Such a concentration of ammonia is sufficient to sustain some phytoplankton growth in summer, nitrogen-depleted seawater (Bougis 1974; Parsons et al. 1977).

The results in Table 4 suggest that incubation can increase (at $P < 0.01$, using a two-way Anova on the results of Table 4) the release of nutrients by 20 to 90%, a finding that is consistent with earlier work by

TABLE 3. Amounts of nutrients (in g-at. $\cdot L^{-1}$) released by the bird community over the entire 30.6 km² study area during the summer 1978. The integrated seasonal total (extreme right column) is based upon a 183-d season.

	Census date																		
	May			June				July			Aug.			Sept.		Oct.		Nov.	Total
	17	24	31	7	16	22	29	11	18	28	5	19	28	11	19	4	19	16	(kg-at.)
NH_3	77.1	78.3	66.1	71.1	125.2	251.3	232.9	200.1	217.0	241.9	293.5	317.2	361.3	470.1	486.0	309.1	209.7	22.9	43.7
NO_2^{2-}	0.5	0.4	0.3	0.3	0.8	2.0	1.8	1.6	1.7	2.0	2.3	2.6	3.0	4.0	4.2	2.7	1.7	0.2	0.35
NO_3^{3-}	6.5	5.7	4.3	4.6	9.9	22.8	21.3	18.5	19.5	22.5	26.7	29.7	34.2	45.8	47.4	30.1	20.2	1.9	4.1
N_{tot}	84.1	84.4	70.7	76.0	135.8	276.0	256.0	220.2	238.7	266.3	322.6	349.5	398.4	519.9	537.5	341.9	231.6	24.9	48.1
SiO_3^{2-}	9.1	7.2	5.0	5.3	13.1	32.6	30.8	27.0	28.1	32.6	38.3	43.4	50.0	68.2	70.7	45.1	29.8	2.6	5.8
PO_4^{3-}	10.5	15.2	15.4	16.4	20.4	27.2	24.2	19.5	23.9	23.8	31.4	29.7	32.4	35.4	36.2	22.7	17.1	3.5	4.2

TABLE 4. Changes in the contents of soluble nitrogen in the excreta (μg-at.$\cdot L^{-1}\cdot g^{-1}$ of excreta) during incubation at ambient temperature for 2 d (see text).

	Incubation time (h)						
	0	8	16	24	32	40	48
Common eider (*Somateria mollissima*)	44	50	70	71	79	84	75
Herring gull (*Larus argentatus*)	1398	1324	1329	1516	1644	1692	1853
Great black-backed gull (*Larus marinus*)	1314	1217	1205	1255	1197	1356	1452
Ring-billed gull (*Larus delawarensis*)	1846	1695	1802	1915	1965	1904	2037

Copeman and Dillman (1937). Hence, all the values given so far could be raised by this amount when the 2-d period following release is considered. Beyond this, more, and eventually all of the initially nonsoluble excreta will be released through decomposition.

The role of birds in nutrient cycling in the coastal waters of the St. Lawrence estuary may now be assessed. Other aspects of this research program (J. C. Therriault et al. unpublished data) have confirmed that, except for a few weeks in the summer where some nitrogen depletion was recorded, nutrients in the surface layers of the coastal study area are generally not limiting for production. We consider that at least two sources of biogenic elements are much more important in the nutrient budget of the coastal waters of the Métis study area. The first is land drainage and a brief example will underline the magnitude of its impact. One of the freshwater rivers entering the study area is the moderately polluted Mitis river that carries over a single July day, ~75 kg-at. of soluble nitrogen[1] (sum of NH_3, NO_3^{3-}, and NO_2^{2-}) while the entire bird population excretes about 0.24 kg-at. of these nutrients during the same period.

Another source probably ranking higher still in importance in the coastal nutrient budget is vertical mixing. Because of the physical conditions prevailing in the region (strong tidal influence, periodical windstorms, and presence of numerous shoreline discontinuities), the circulation pattern in the study area is characterized by regular vertical turbulent mixing. Available data on the vertical distribution of nutrients at a near (250 m) shore station off Pointe Métis reveal that even though partial depletion may temporarily occur in the uppermost 3 m of the water column, high concentration of N is always present below; thus, nitrate (in μg-at.$\cdot L^{-1}$) at 0, 1, 3, and 5 m read 0.4, 0.8, 4.6, and 4.1, respectively, on July 13, 1978; 2.3, 2.4, 4.3, and 5.8 on July 26, 1978; 1.2, 0.8, 1.0, and 8.5 on August 9, 1978; 16.0, 16.0, 16.2, and 16.2 on March 23, 1979; 1.4, 0.4, 1.7, and 6.7 on June 8, 1979; and finally, 10.0, 8.9, 10.5, and 8.9 on August 22, 1979 (J. C. Therriault et al. unpublished data). The average daily contribution of the birds (0.02 μg-at.$\cdot L^{-1}$, see above) remains 20 to 50 $\times$ smaller than the existing nitrate levels in the partially depleted 0- to 3-m surface layer (June and July). Thus, even a relatively mild wind event could lead to the introduction in the surface waters, through vertical mixing, of many times more nutrients in a very short period of time than the entire bird population could add to this layer over the entire season.

Had we considered that the nutrients released by the birds were introduced in the uppermost 5 m of the water column, instead of the uppermost 1 m as we have done, the overall contribution by these animals to the nutrient budget of these waters would have lost further significance. Even a consideration of the fact that only about one fifth of the excreta is initially soluble and that decomposition will eventually free all the remaining nutrients does not alter substantially the overall picture.

We therefore conclude that the bird community has little importance, if any, in replenishing nutrients in the surface waters of the littoral zone in the St. Lawrence Estuary, with the possible exception of a very localized influence around densely populated nesting islands (such as Ile-Aux-Pommes or Bicquette Island: see Reed 1975) or in the extremely narrow inshore area where the birds sometimes aggregate for feeding or resting, especially during the short period of nutrient depletion in the estuary. We conclude that the seabirds play only a minor role in the dynamic process of the nutrient regeneration in this coastal environment. We see the bird community instead as superimposed upon the marine ecosystem and living off excesses in production of the latter without really influencing it. Considering the Gulf of St. Lawrence as a whole, in which the entire bird population has been estimated at 400 000 (Steven 1975), we are also tempted to reach the same conclusion.

Our results confirm an earlier demonstration by Cantin et al. (1974) that underlined the role of the common eiders in milling huge quantities of mussel material every day. They estimated that the St. Lawrence Estuary population grinds between 40 and 100 t of mussels and periwinkles (*Littorina* spp.) every day amounting to the impressive seasonal total of well over 10 000 t. Considering that this process takes place in a very narrow intertidal zone, it cannot be lightly discarded. Such a rapid turnover of both organic and inorganic material must be considered important with respect to the population biology of the molluscs involved and to the ecology of the intertidal zone as well. But these figures, impressive as they may be, are dwarfed by the magnitude of the processes that take place in the inshore waters of the estuary and Gulf of St. Lawrence as a whole.

[1]Mitis river flow taken as 17 000 L$\cdot s^{-1}$ and soluble N compounds as 0.5 μg-at.$\cdot L^{-1}$ (Comité d'Etude sur le Fleuve Saint-Laurent 1978).

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BELOPOL'SKII, L. O. 1961. Ecology of sea colony birds of the Barents Sea. (Transl. from Russian by Israel Program for Sci. Transl., Jerusalem.)

BOUGIS, P. 1974. Ecologie du plancton marin. 1. Le phytoplancton. Masson et Cie., Paris.

CANTIN, M., J. BÉDARD, AND H. MILNE. 1974. The food and feeding of common eiders in the St. Lawrence estuary in summer. Can. J. Zool. 52: 319–334.

COMITÉ D'ÉTUDE SUR LE FLEUVE SAINT-LAURENT. 1978. Rapport d'étude sur le tronçon en aval de Montmagny. Vol. 5. p. 885–1453. Services de Protection de l'Environnement, Qué. (Mimeogr.)

COPEMAN, P. R., AND F. J. DILLMAN. 1937. Changes in the composition of guano during storage. J. Agric. Sci. 27: 178–187.

GALKINA, V. N. 1974. Chemical composition of soluble substances of excreta of fish-eating sea birds. Ekologiya 5: 23–28.

1977. Effect of soluble organic compounds of excrements of marine colonial birds on phytoplankton photosynthesis. Ekologiya 8: 77–82.

GAUTHIER, J., AND J. BÉDARD. 1976. Les déplacements de l'eider commun dans l'estuaire du Saint-Laurent. Nat. Can. 103: 161–191.

GOLOVKIN, A. N. 1963. Fish consumption by guillemots and kittiwakes in the nesting period in the Barents Sea. Zool. Zh. 62: 408–416.

1967. The effect of colonial sea birds on the development of phytoplankton. Okeanologiya 7: 672–682.

1976. Bird bazaars in the north of Novaya Zemlya, p. 90–98. *In* Peculiarities of biological productivity of waters near bird's bazaars in the north of Novaya Zemlya. National Science Foundation, Washington, D.C., United States Department of Commerce, Publication PB-267, 499-T.

GOLOVKIN, A. N., AND G. P. GARKAVAYA. 1975. Fertilization of waters of the Murmansk coast by bird excreta near various types of colonies. Biol. Morya 5: 49–57.

GOLOVKIN, A. N., AND V. I. GUREVICH. 1974. Determination and estimation of hydrochemical anomalies in the sea using the method of linear discriminant functions (exemplified by seashore colonies in the Barents Sea). Okeanologiya 13: 804–808.

GOLOVKIN, A. N., AND L. E. POZDNYAKOVA. 1966. Specific characteristics and scale of effect of colonial sea birds on the regime of biogenic salts in Murmansk coastal waters, p. 210–230. *In* Fish-eating birds and their significance in the national economy. Nauka, Moscow.

GOLOVKIN, A. N., AND E. A. ZELICKMAN. 1965. The development of *Calanus* in the nesting area of sea bird populations of the Murman coastal sites. Okeanologiya 1: 117–128.

GOLOVKIN, A. N., V. N. SHIROKOLOBOV, AND G. P. GARKAVAYA. 1972. Characteristics of distribution of biogenic elements in the region of bird rookeries of northern Novaya Zemlya, p. 33–46. *In* Characteristics of the biological productivity of waters near bird rookeries in Novaya Zemlya. Nauka, Leningrad.

HUTCHINSON, G. E. 1950. Biogeochemistry of vertebrate excretion. Bull. Am. Mus. Nat. Hist. 96: 1–554.

KING, J. R. 1974. Seasonal allocation of time and energy resources in birds, p. 4–85. *In* R. A. Paynter Jr. [ed.] Avian energetics. Publications of the Nuttall Ornithological Club, Cambridge, Mass.

LASIEWSKI, R. C., AND W. R. DAWSON. 1967. A re-examination of the relation between standard metabolic rate and body weight in birds. Condor 69: 13–23.

OWEN, M. 1975. An assessment of fecal analysis technique in waterfowl feeding studies. J. Wildl. Manage. 39: 271–279.

PARSONS, T. R., M. TAKAHASHI, AND B. HARGRAVE. 1977. Biological oceanographical processes. 2nd ed. Pergamon Press, New York, N.Y.

REED, A. 1975. Les colonies d'oiseaux aquatiques dans l'estuaire du Saint-Laurent. Faune du Québec Bull. 19: 76 p.

RYDER, J. P. 1978. Sexing ring-billed gulls externally. Bird-Banding 49: 218–222.

STEVEN, D. M. 1974. Primary and secondary production in the Gulf of St. Lawrence. Mar. Sci. Cent. (McGill Univ.) Rep. 26: 1–116 p.

1975. Biological production in the Gulf of St. Lawrence, p. 229–248. *In* T. W. M. Cameron and L. W. Billingsley [ed.] Energy flow, its biological dimension: a summary of the IBP in Canada, 1964–74, Ottawa.

STRICKLAND, J. D. H., D. HOLM-HANSEN, R. W. EPPLEY, AND R. J. LINN. 1969. The use of a deep tank in plankton ecology. 1. Studies of the growth and composition of phytoplankton crops at low nutrients levels. Limnol. Oceanogr. 14: 23–24.

STRICKLAND, J. D., AND T. R. PARSONS. 1972. A practical handbook of seawater analysis. 2nd ed. Bull. Fish. Res. Board Can. 167: 310 p.

THRELFALL, W., AND D. D. JEWER. 1978. Notes on the standard body measurements of two populations of herring gulls (*Larus argentatus*). Auk 95: 749–753.

TUCK, L. M. 1961. The murres: their distribution, populations and biology. Can. Wildl. Serv., Ottawa.

USPENSKI, S. M. 1959. Colonially nesting sea birds of the northern and far eastern seas of the USSR, their location, numbers and role as consumers of plankton and benthos. Byull. Mosk. Ova. Ispyt. Prir. Otd. 44: 39–52.

ZELICKMAN, E. A., AND A. N. GOLOVKIN. 1972. Composition, structure and productivity of neritic plankton communities near the bird colonies of the northern shores of Novaya Zemlya. Mar. Biol. 17: 265–274.

Nutrient Distributions in the Canadian Archipelago: Indicators of Summer Water Mass and Flow Characteristics

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JONES, E. P., AND A. R. COOTE. 1980. Nutrient distributions in the Canadian Archipelago: indicators of summer water mass and flow characteristics. Can. J. Fish. Aquat. Sci. 37: 589–599.

Concentrations of the nutrients, phosphate, nitrate, and silicate, were measured at several locations in Lancaster Sound, Jones Sound, and Smith Sound. Arctic Ocean surface layer water flowing out through Lancaster Sound and Jones Sound have higher phosphate and silicate concentrations than that flowing out through Smith Sound. Phosphate and silicate concentrations can be useful to indicate flow patterns within some regions of the Canadian Archipelago and to delineate partially the gyre in the Canadian Basin of the Arctic Ocean.

Key words: nutrients: phosphate, nitrate, silicate; distribution: Arctic, Canadian Archipelago; water mass, identification, mixing

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Les concentrations d'éléments nutritifs, phosphates, nitrates et silicates, ont été mesurées à plusieurs endroits dans les détroits de Lancaster, Jones et Smith. L'eau de la couche superficielle de l'océan Arctique sortant par les détroits de Lancaster et Jones ont des teneurs en phosphates et silicates plus élevées que celle sortant par le détroit de Smith. Les concentrations de phosphates et de silicates peuvent être d'utiles indicateurs des caractéristiques de l'écoulement à l'intérieur de certaines régions de l'Archipel canadien et un moyen de délimiter partiellement le tourbillon dans le bassin canadien de l'océan Arctique.

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THE water which flows through the Canadian Archipelago originates in the surface layer of the Arctic Ocean. According to Coachman and Aagaard (1974), this water can be described as follows. Bering Sea water flowing through Bering Strait over a sill of 45 m and North Atlantic water entering the Arctic Ocean at mid-depth combine to form the Arctic Ocean surface layer which is about 200 m deep. Clearly identifiable Bering Sea water has only occasionally been encountered east of 145°W and then only as an isolated patch. The surface layer water flows out of the Arctic Ocean through the Canadian Archipelago over sills 130 m deep in Lancaster Sound, 150 m deep in Fram Sound (at the western end of Jones Sound), and 250 m deep in Nares Strait. It also flows out of the Arctic Ocean through Fram Strait to the Greenland Sea.

Few measurements of nutrient concentrations relevant to studies of the Canadian Archipelago have been made. An extensive study of nutrients in the East Siberian and Laptev seas was made by Codispoti and Richards (1968). Kinney et al. (1970) report chemical characteristics of Arctic Ocean water measured from an ice station north of Point Barrow, and Codispoti (1968) reports nutrient concentrations in the Greenland Sea. Codispoti and Owens (1975) report measurements made across one section in Lancaster Sound. McGill and Corwin (1964) report a number of measurements extending from Kane Basin to northern Baffin Bay. These measurements were made under the unusual circumstance of a large ice-island plug in Kennedy Channel, producing open conditions in Kane Basin, and were made under more or less ice-free summer conditions. In the study to be reported here, these measurements have been extended considerably in Lancaster Sound and Jones Sound and supplement those in Smith Sound, ours being obtained under more normal summer conditions. This paper focuses on our measurements of nutrients: phosphates, nitrates, and silicates, and what they show regarding the character of the water within the Canadian Archipelago and the circulation of the surface layer of the Arctic Ocean.

Methods

In an attempt to characterize the origin and flow of the Arctic Ocean surface water through the Canadian Archipelago itself, we collected samples at several stations

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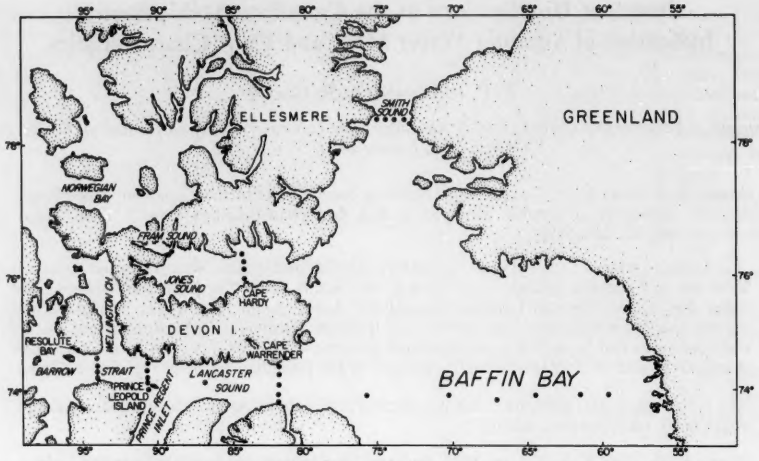

FIG. 1. Schematic map of the Canadian Archipelago showing the locations of stations.

throughout the Canadian Archipelago and Baffin Bay on a cruise of the CSS *Hudson* during August and September 1977 (Fig. 1).

Nutrient concentrations were determined within a few hours of sample collection using standard methods with an AutoAnalyzer II. The analytical precision was 2% and accuracy better than 5%. Other determinations relevant to the interpretation of the nutrient results were temperature values measured with a Guildline Mk. III analogue CTD system. Temperatures were also measured at selected depths using both protected and unprotected reversing thermometers to check the CTD results. Salinity values were determined in samples from all sample depths using a Guideline Autosal model 8400 salinometer.

Results and Discussion

A major goal of this work is to examine Arctic Ocean surface water flowing through Lancaster Sound, Jones Sound, and Smith Sound with a view to characterizing water masses and hence circulation for summer conditions. To help discuss water masses in the Canadian Archipelago, we will follow the labels and descriptions used by Muench (1971). The two main water types in this region are Arctic Ocean water from the Arctic Ocean surface layer and Baffin Bay Atlantic water from the Atlantic Ocean which has entered Baffin Bay through Davis Strait. Arctic Ocean surface layer water has relatively low salinity values, from about 32‰ up to about 34‰, and is cold, with temperatures below 0°C. Baffin Bay Atlantic water has higher salinity values, near 34.4‰, and is generally warmer, from 0 to 2°C and higher.

Lancaster Sound

Three sections were occupied in Lancaster Sound. The most western section near Resolute Bay is about 100 km east of the sill in Barrow Strait. A second section was chosen to be near Prince Leopold Island, the location of an especially important bird colony. This section also corresponds fairly closely to one chosen by Codispoti and Owens (1975) for measurements of nutrient concentrations. The third section is at the eastern entrance to Lancaster Sound off Cape Warrender on Devon Island.

Our salinity and temperature values for these sections show distributions similar to those reported earlier (Collin 1962). From these measurements, the general picture of water in Lancaster Sound is that of Arctic Ocean surface layer water flowing through Barrow Strait and Wellington Channel and mixing with Baffin Bay Atlantic water. Within Lancaster Sound, the eastward current flows along the south side. There is also a westward current flowing along the north side from Baffin Bay to a point west of Prince Regent Inlet but not so far as Wellington Channel (Bailey 1957; Collin 1962).

At our Resolute Bay section (Fig. 2), there is no indication of warmer, more saline Baffin Bay Atlantic water or a mixture of it with Arctic Ocean water. Above about 50 m, the water is even less saline because of melting sea ice and runoff, and it is also warmer, as one would expect from summer heating. The nutrient concentrations show low values at the surface, resulting primarily from biological depletion, building to higher values at about 100 m. Since the salinity and temperature values show no evidence of Baffin Bay Atlantic water at this section, we presume that the nutrient concentrations below depths where depletion from biological activity occurs represent concentrations in water flowing from the Arctic Ocean. Station 33, the most northern station in this section, has a much smaller nutrient concentration increase with depth than do the

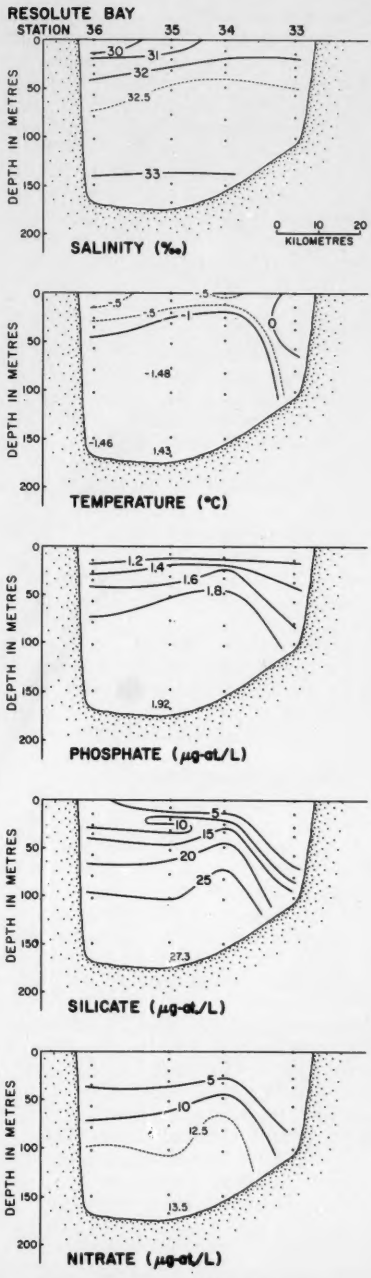

FIG. 2. Resolute Bay section: salinity, temperature, phosphate, silicate, and nitrate. Station locations 33: 74°36.6′N, 94°20.4′W; 34: 74°27.7′N, 94°16.2′W; 35: 74°20.0′N, 94°15.8′W; 36: 74°10.7′N, 94°14.6′W.

others in this section. This is reflected also in temperatures and oxygen concentrations (not shown), but not in salinity values. We have no ready explanation for these observations. Bailey (1957) and Collin (1962) both show maps of computed surface currents in which a current through Wellington Channel branches near Cornwallis Island to flow both east and west in Lancaster Sound, indicating Station 33 could be different from others in the section. Perhaps the values at Station 33 result from some kind of interaction between easterly and westerly currents in this section, although a plot of density, which closely resembles a plot of salinity at these temperatures, shows no indication of geostrophic shear. Unfortunately, we do not have nutrient concentration measurements in Wellington Channel but we would not expect them to be much different from those in the Fram Sound (Fig. 6) and Resolute Bay sections.

In the Prince Leopold Island section (Fig. 3) we judge the water down to the temperature minimum to be from the Arctic Ocean. Below that depth, the salinity increases to values higher than observed in the Resolute Bay section. The temperature also increases to higher values than the deeper water in the Resolute Bay section, but neither reaches values associated with Baffin Bay Atlantic water. Thus we conclude that this water is a mixture of Arctic Ocean and Baffin Bay Atlantic waters. The nitrate concentrations are similar to those in the Resolute Bay section. The phosphate and silicate concentrations are also similar above about 100 m but become distinctly lower at depths below 100 m, about where the temperature and salinity values indicate mixing with Baffin Bay Atlantic water. The nutrient values in this section are quite similar to those reported by Codispoti and Owens (1975) for a nearby section.

In the Cape Warrender section (Fig. 4), Baffin Bay Atlantic water is clearly evident below about 250 m. The region of the temperature minimum would seem to be mixed Baffin Bay Atlantic water and Arctic Ocean water. Nearer the surface, salinity values are a little higher than the Resolute Bay and Prince Leopold Island sections and the temperatures, especially above 50 m, are warmer. The nitrate concentrations and salinities are roughly the same at similar depths in the Prince Leopold Island section (Fig. 3), but increase below 250 m to the bottom at about 800 m. In contrast, both the phosphate and silicate concentrations show a smaller concentration gradient in this section than in the Prince Leopold Island section.

The striking difference in nutrient concentration gradients in Lancaster Sound is illustrated in Fig. 5 which shows a west–east section from Resolute Bay across northern Baffin Bay to Greenland. The phosphate and silicate concentrations especially show that the water at the Cape Warrender section is very different from that in western Lancaster Sound. The

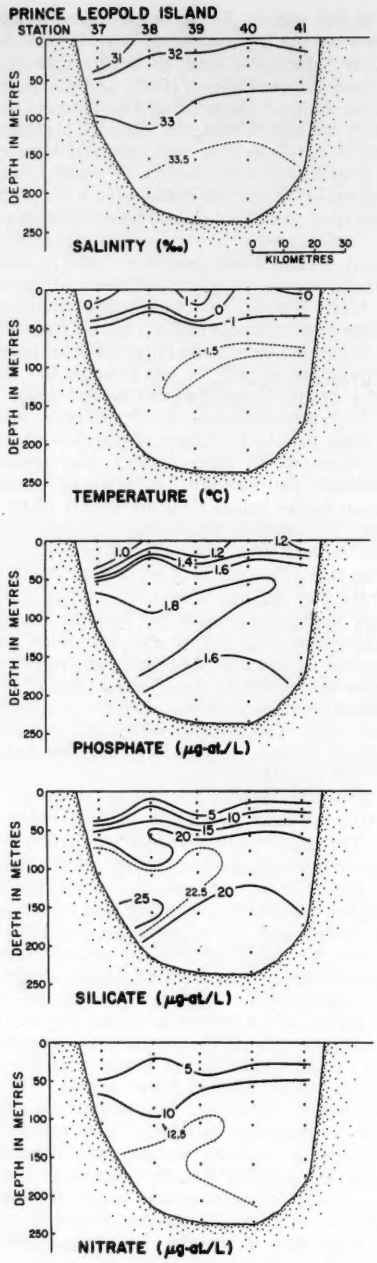

FIG. 3. Prince Leopold Island section: salinity, temperature, phosphate, silicate, and nitrate. Station locations 37: 73°58.7′N, 90°22.9′W; 38: 74°7.1′N, 90°22.6′W; 39: 74°15.3′N, 90°25.9′W; 40: 74°24.1′N, 90°22.2′W; 41: 74°33.0′N, 90°22.8′W.

Arctic Ocean water entering Lancaster Sound from the west seems to lose its identity somewhere near Station 42, north of the Brodeur Peninsula. At the Cape Warrender section, the surface water has temperature and salinity values typical of the Arctic Ocean, but nutrient concentrations in both the surface water and the deeper water very closely resemble water at similar depths in northern Baffin Bay. Note that in this and almost all the other nutrient plots, nutrient concentrations near the surface are reduced because of biological activity. Different water flow at the surface is not implied by this.

JONES SOUND

Two sections were occupied in the Jones Sound region: one across Fram Sound through which Arctic Ocean water flows into the western end of Jones Sound (Fig. 6), and one slightly to the east of the centre of Jones Sound, the southernmost station (Station 47) being close to Cape Hardy (Fig. 7). Less work has been reported for Jones Sound than Lancaster Sound. Brief discussions of this region based on salinity and temperature measurements are provided by Bailey (1957), Collin and Dunbar (1964), and Coachman and Aagaard (1974). Some more recent results are discussed by Barber and Huyer (1977).

Compared to the Resolute Bay section in Lancaster Sound, the salinity values in Fram Sound (Fig. 6) towards the bottom are high and the temperatures are warm, but they still lie in the range expected for Arctic Ocean surface water (Collin and Dunbar 1964). Nutrient concentrations are in general similar to or perhaps slightly lower than those in the Resolute Bay section of Lancaster Sound.

In the Cape Hardy section (Fig. 7) the surface salinity values are similar to those in the Fram Sound section but become considerably higher near the bottom at 600 m. These deeper values are comparable to the salinity values in Baffin Bay at similar depths but the water is not quite so saline as Baffin Bay Atlantic water. Surface temperatures are similar to those in the Fram Sound section. The deeper water has higher temperatures, up to nearly 0°C at 600 m, similar to those in Baffin Bay at these depths, but again not quite so warm as Baffin Bay Atlantic water. The nutrients in general have concentrations similar to those in Fram Sound at comparable depths, but increase towards the bottom and are not unlike those of Baffin Bay down to about 600 m (Fig. 5). There is a sharp minimum in the nutrient concentrations, especially for phosphate and silicate, at about 200 m. This indicates a sharper demarcation between Arctic

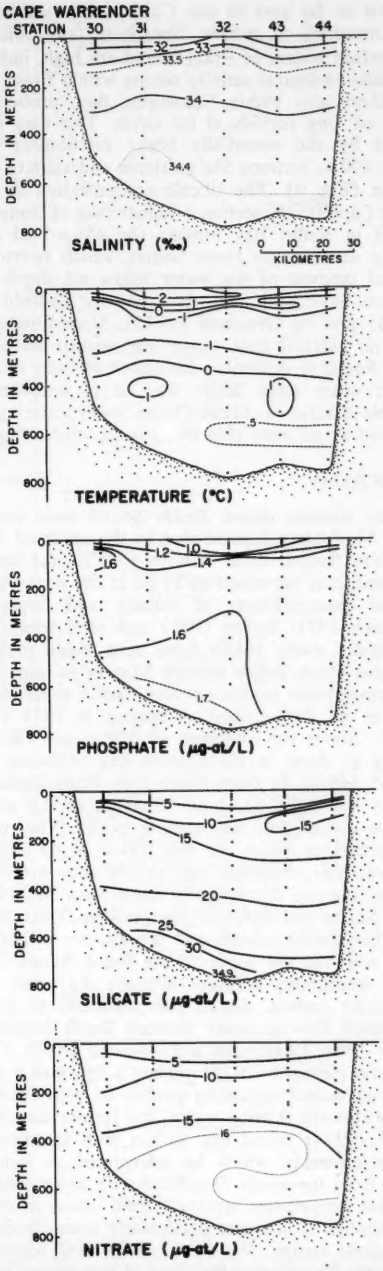

FIG. 4. Cape Warrender section: salinity, temperature, phosphate, silicate, and nitrate. Station locations 30: 73°47.1′N, 81°57.7′W; 31: 73°55.0′N, 82°00.0′W; 32: 74°09.4′N, 81°58.9′W; 43: 74°18.0′N, 81°57.7′W; 44: 74°26.3′N, 81°58.3′W.

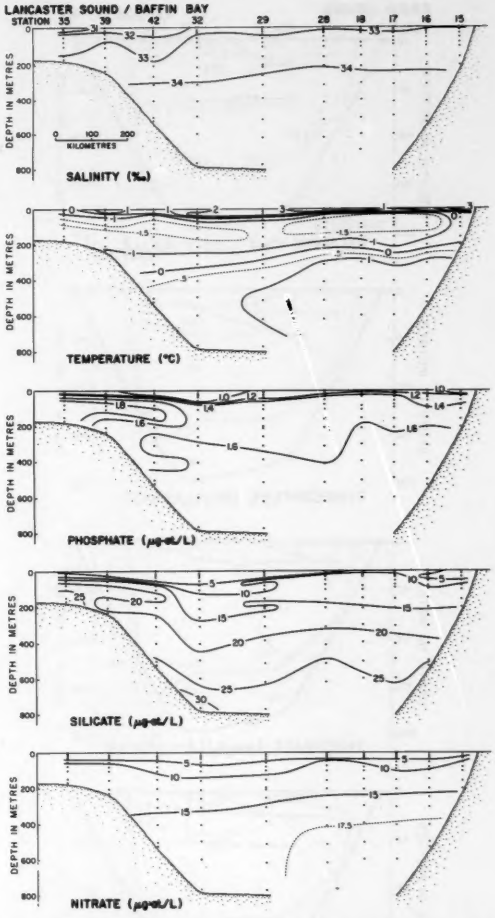

FIG. 5. Resolute Bay to Greenland section: salinity, temperature, phosphate, silicate, and nitrate. Station locations 35: 74°20.0′N, 94°15.8′W; 39: 74°15.3′N, 90°25.9′W; 42: 74°10.9′N, 85°58.5′W, 29: 73°42.0′N, 75°55.0′W; 28: 73°42.0′N, 70°29.0′W; 18: 73°27.3′N, 67°17.3′W; 17: 73°42.0′N, 64°34.9′W; 16: 73°41.2′N, 61°38.6′W; 15: 73°41.8′N, 58°39.5′W.

Ocean and Baffin Bay waters than observed in Lancaster Sound.

Although our data are less abundant for Jones Sound than Lancaster Sound, the evidence is reasonably clear that Arctic Ocean water enters Jones Sound from Fram Sound and overlies water from Baffin Bay

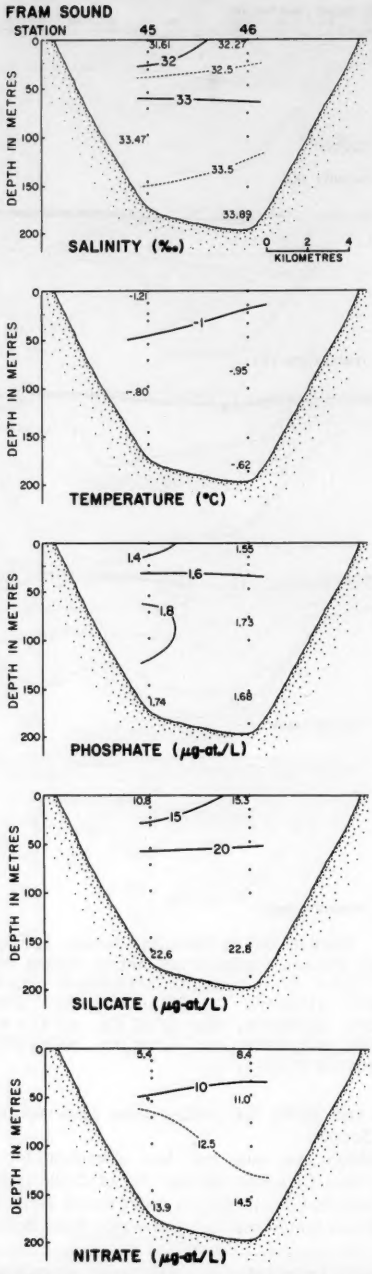

FIG. 6. Fram Sound section: salinity, temperature, phosphate, silicate, and nitrate. Station locations 45: 76°21.1′N, 89°25.8′W; 46: 76°23.6′N, 89°18.3′W.

at least as far east as our Cape Hardy section. The concentrations of nitrate, phosphate, and silicate in the surface waters of Fram Sound are high, indicating that little biological activity occurs within Fram Sound and likely also within Norwegian Bay, probably because of long periods of ice cover. This idea is supported by the essentially linear relationship above about 100 m between the nutrients and salinity in this section (Fig. 9). The silicate concentration at 660 m in the Cape Hardy section is higher than at comparable depths in Baffin Bay outside the 450-m sill at the eastern entrance to Jones Sound, which restricts the rate of renewal of the water below sill depth. Such features have been fully described by Redfield et al. (1963) and by Grasshoff (1975). Unfortunately, we have no nutrient data nearer the eastern entrance of Jones Sound to determine the extent of entry of Arctic Ocean water from Baffin Bay or to document the possible demise of Arctic Ocean water entering from the west as we were able to do in Lancaster Sound.

SMITH SOUND

Four stations across Smith Sound were occupied (Fig. 8). Although somewhat to the south of the sill in Smith Sound, these stations were as far north as ice conditions permitted us to go at the time. A number of measurements of salinity and temperature (Muench 1971; Sadler 1976) and of nutrients (McGill and Corwin 1964) have been made previously in Nares Strait. Below the top 50 m or so, our salinity and temperature results are very similar to those measured at an almost identical section in 1971 (Sadler 1976). Above the sill depth of 250 m our values are similar to those in the Lincoln Sea (Muench 1971; Siebert 1968). In comparison with Fram Sound, the salinity values below about 50 m are similar at comparable depths, but the water is colder. The nutrient concentrations below the top 100 m or so are fairly uniform. The phosphate and silicate concentrations in this section are dramatically lower than those in the Fram Sound and Resolute Bay sections. Thus, in spite of rather similar salinity and temperature values in all three sections, the water of the Smith Sound section has a measurably different nutrient character.

Various authors discuss the possibility of episodic northward flow of water through Smith Sound (e.g. Sadler 1976; Coachman and Aagaard 1974). Coachman and Aagaard (1974) present a figure of a section in Smith Sound indicating parcels of both southward and northward flowing water, the former dominating. Sadler (1974) found one station with an anomalous T–S relationship which he interpreted as indicating water from the south. Our Station 55 has a somewhat different temperature structure from others in the section, though it does not particularly resemble Sadler's anomalous station. We cannot draw firm conclusions about the flow during the time of our cruise, but the salinity and temperature values are characteristic of water from the Lincoln Sea and, except for slightly

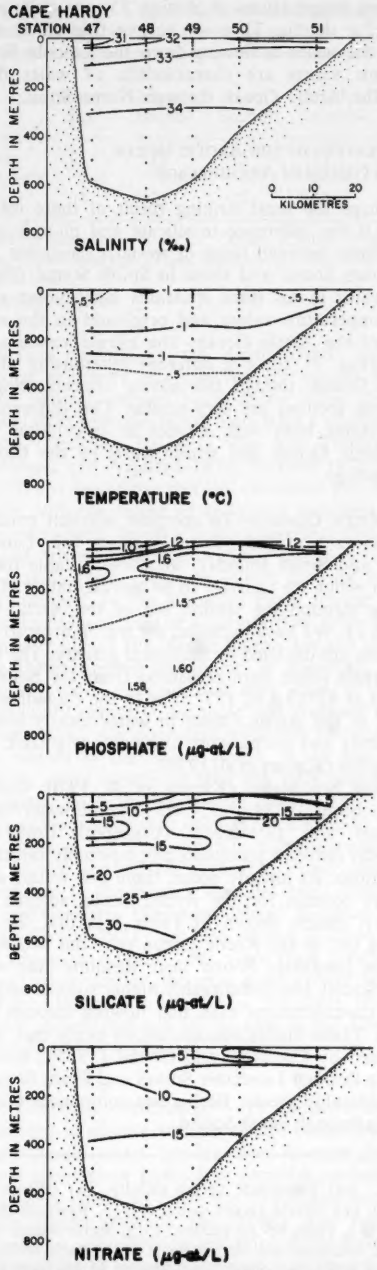

FIG. 7. Cape Hardy section: salinity, temperature, phosphate, silicate, and nitrate. Station locations 47: 75°51.8′N, 83°49.9′W; 48: 75°58.0′N; 83°50.5′W; 49: 76°4.7′N, 83°49.9′W; 50: 76°10.0′N, 83°51.4′W; 51: 76°20.1′N, 83°49.3′W.

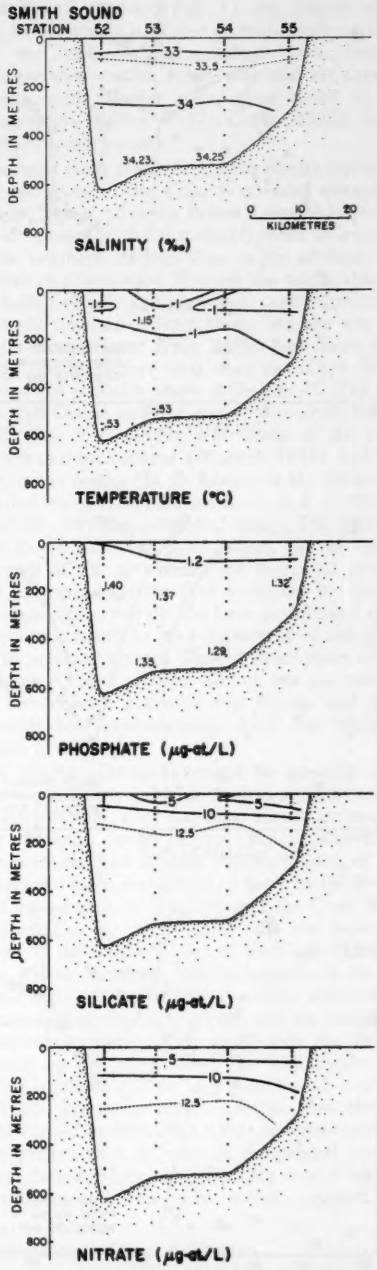

FIG. 8. Smith Sound section: salinity, temperature, phosphate, silicate, and nitrate. Station locations 52: 78°19.2′N, 74°43.0′W; 53: 78°18.9′N, 74°16.0′W; 54: 78°20.4′N, 73°39.8′W; 55: 78°19.0′N, 73°5.3′W.

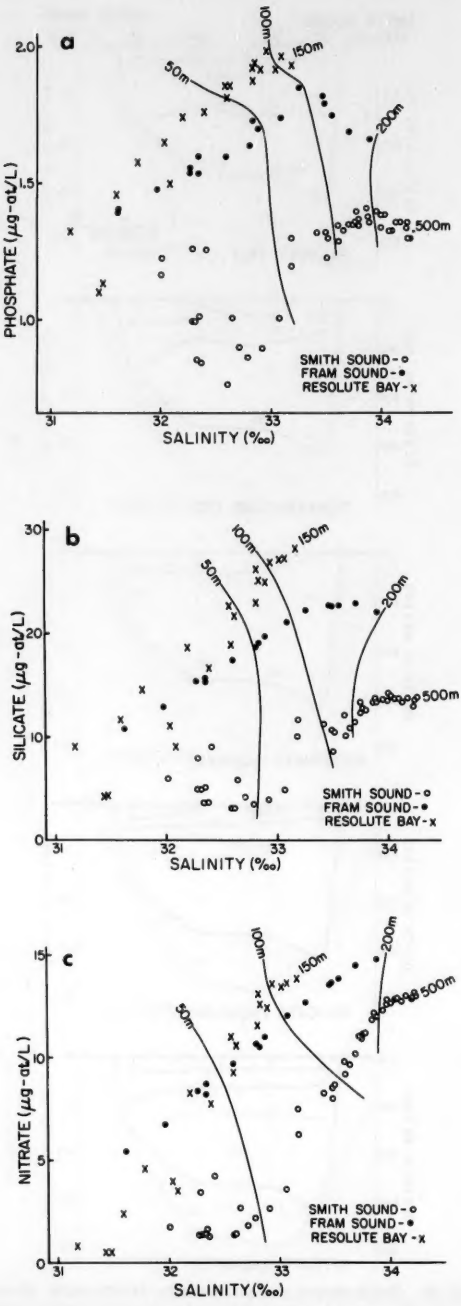

FIG. 9. (a) Phosphate versus salinity, (b) silicate versus salinity, (c) nitrate versus salinity. (○), Resolute Bay section; (●), Fram Sound section; (×), Smith Sound section. Depths indicated are approximate since each section plot includes more than one station. Station 33 has been omitted from the Resolute Bay section because of its anomalous values.

different temperatures at Station 55, are quite similar at similar depths. Thus we believe that all or almost all of the water is flowing from the Lincoln Sea and that our values are characteristic of water flowing from the Arctic Ocean through Nares Strait.

CIRCULATION IN THE ARCTIC OCEAN AND CANADIAN ARCHIPELAGO

Perhaps the most striking result of these measurements is the difference in silicate and phosphate concentrations between those in western Lancaster Sound and Fram Sound and those in Smith Sound (Fig. 9). The water in all three locations has similar salinity and temperature values and originates in the surface layer of the Arctic Ocean. The nitrate versus salinity plots (Fig. 9) show a different relationship also for Smith Sound, though the nitrate concentrations for the three sections are very similar. This difference has implications both with respect to circulation within the Arctic Ocean and within parts of the Canadian Archipelago.

1. Arctic Ocean — To compare nutrient concentrations in each of these three locations (i.e. Lancaster, Fram, and Smith sounds), we chose a value for each section which we consider to be representative of water flowing through the section out of the Arctic Ocean (Table 1). We have excluded the top 50 m or so where nutrients are depleted by biological activity. The Bering Sea values listed were measured from Ice Station T3 located at 85°15.8′N, 157°28′W in the Canadian Basin region of the Arctic Ocean in water clearly identified by salinity and temperature as having originated in the Bering Sea (Kinney et al. 1970).

Bering Sea water (Kinney et al. 1970; Codispoti and Richards 1968) and North Atlantic water between Svalbard and Greenland (Codispoti 1968) have markedly different phosphate and especially silicate concentrations. As already noted, these two waters are the primary sources for the Arctic Ocean surface layer. What is clearly shown in Table 1 is that the water flowing out of the Arctic Ocean over the two western sills, in Lancaster Sound near Resolute Bay and in Fram Sound, has considerably higher silicate and phosphate concentrations than that flowing through Smith Sound. These higher concentrations imply that, as first suggested by Codispoti and Owens (1975), the water flowing through Lancaster Sound and Fram Sound has a considerably greater Bering Sea component than that flowing through Smith Sound.

TABLE 1. Average silicate concentrations for water flowing into and out of the Arctic Ocean (μg-at.$\cdot$L^{-1}).

	Bering Sea[a]	Lancaster Sound[b]	Fram Sound[b]	Smith Sound[b]	Northern Greenland Sea[c]
Nitrate	15	12	13	13	10
Phosphate	2.0	1.7	1.7	1.3	0.8
Silicate	34	25	22	11	5

[a]Bering Sea water in Arctic Ocean, Kinney et al. (1970). Approximate average values between 100 and 200 m.
[b]This work.
[c]Codispoti (1968).

Surface currents in the Arctic Ocean have been mapped primarily by observed ice station and ship drifts. Several authors have drawn maps showing a general drift over the North Pole with a clockwise gyre in the Canadian Basin (Collin and Dunbar 1964; Coachman and Aagaard 1974; Pickard 1975). Campbell (1965) has shown that this clockwise gyre coincides closely with the center of the mean atmospheric pressure anticyclone, leading to the suggestion that the gyre is wind driven. Although evidence for the existence of the gyre is fairly clear, its extent has not been precisely defined. In the Eurasian Basin similar observations lead to a picture of a surface flow from the whole Eurasian side of the Arctic Ocean toward the North Pole. After passing the region of the Pole, the flow becomes more concentrated and exits as the East Greenland Current (Coachman and Aagaard 1974).

Our results are consistent with this surface circulation pattern in the Arctic Ocean. What they seem to indicate more clearly is that the near surface water associated with the clockwise gyre in the Canadian Basin extends at least as deep as the sill depths (150 m) of Lancaster and Fram Sounds. Silicate concentrations just outside the sill of d'Iberville Fiord which is an appendage to the Nansen Sound–Greely Fiord system on northwestern Ellesmere Island (Frozen Sea Research Group 1977) are also similar to those in Fram and Lancaster sounds. Thus, since these concentrations are all quite similar, the gyre must extend as far east as Peary Channel in the Queen Elizabeth Islands and Nansen Sound but not so far east as the Lincoln Sea which flows in part into Smith Sound.

2. Canadian Archipelago — In Lancaster Sound the difference in silicate and phosphate concentrations between Arctic water flowing from Barrow Strait and Arctic water from Baffin Bay provides an independent and better signal to trace the circulation within Lancaster Sound than do the salinity and temperature values. The water flowing east through the Resolute Bay section (Fig. 2) represents a source of these higher nutrient concentrations, while the water entering from Baffin Bay has lower concentrations. In the Prince Leopold Island section (Fig. 3) the silicate concentration in particular is higher at mid-depths in the southern part than in the northern part, indicating a westerly flow on the north side and an easterly flow on the south. Earlier calculations based on dynamic topography (Bailey 1957; Collin 1962) indicate a similar current pattern.

Perhaps more dramatic are the silicate and phosphate concentrations at the Cape Warrender section (Fig. 4 and 5). Again, as in the Prince Leopold Island section, we see generally lower concentrations at many depths in the northern stations than in the southern stations, indicating a westward flow on the north side towards the higher silicate and phosphate concentrations and an eastward flow away from them. We see also that the Arctic Ocean water from Baffin Bay flows into Lancaster Sound farther west than our Cape Warrender section but probably short of Station 42. The existence of Arctic Ocean water in eastern Lancaster Sound from Baffin Bay is consistent with some of the calculated surface current patterns (Muench 1971) and with surface drifter tracks (D. D. Lemon et al., Arctic Sciences Limited, 9860 West Saanich Road, R.R. 2, Sidney, B.C. V8L 3S1, 1979, unpublished data). The latter show a gyre to the north of Bylot Island, though the surface currents of the gyre seem not to extend quite so far east as the subsurface flow indicated by the nutrient concentrations. We do not have a sufficient density of points to attempt to map the extent of the gyre or to try to produce a more detailed description of the flow patterns within Lancaster Sound, but our results show the considerable potential that silicate and phosphate concentration measurements have for tracing water masses in this region.

A similar discussion might be possible for Jones Sound and Smith Sound if we had a more complete set of nutrient data. Both the Cape Hardy section (Fig. 7) and the Smith Sound section (Fig. 8) show variations in phosphate and silicate concentrations of approximately the same magnitude as those in the Prince Leopold Island section (Fig. 3) and the Cape Warrender section (Fig. 4). One might note the higher silicate values in the northern part of the Cape Hardy section near 200 m, however. This is opposite to the situation at the Prince Leopold Island section where the higher concentrations on the southern end are consistent with a westward current on the north side and an eastward current on the south side. The topography of Jones Sound is different from Lancaster Sound so there is no particular reason to expect a similar flow pattern. Our results could be indicating a gyre or some more complex flow pattern than is found in the central part of Lancaster Sound. More complete data over a wider region would be required to make further comment.

ARCTIC OCEAN SILICATE BUDGET

Codispoti and co-workers have made a case for a net removal of reactive silicate in the Arctic Ocean

TABLE 2. Reactive silicate budget for Arctic Ocean (after Codispoti and Owens 1975, with our values added).

Source	Volume transport (Sverdrups)	Silicate (μg-at.$\cdot$L^{-1})		Silicate transport (μg-at.$\cdot$s$^{-1} \times 10^9$)	
Inflow					
Atlantic water and Arctic bottom water	6	5		30	
Bering Strait	1.5	35		52	
Runoff	0.1	125		12	
Total	7.6			94	
Outflow					
East Greenland current	5.6	8	8	45	45
Canadian Archipelago	2.0	16	21[a]	32	42[a]
Total	7.6			77	87

[a]This work.

(Codispoti and Owens 1975; Codispoti and Lowman 1973). Their conclusions were based on measurements made in Smith Sound during an unusual ice year (McGill and Corwin 1964) and on a section in Lancaster Sound very near to our Prince Leopold Island section.

To determine a silicate budget, they apportioned one-quarter of the estimated flow of two Sverdrups through the Canadian Archipelago to each of Smith and Jones sounds, and one-half to Lancaster Sound. For silicate concentrations they chose values of 10, 15, and 20 μg-at.$\cdot$L^{-1} in Smith, Jones, and Lancaster sounds, respectively. The value for Lancaster Sound, a little lower than what we ascribe to Arctic Ocean water flowing into Lancaster Sound, was measured in a section containing some Baffin Bay water and hence has slightly lower silicate concentrations. In the absence of any measurements at that time in Jones Sound, the value for Jones Sound was taken to be simply the mean of the other two. With these values, they determined the silicate transport through the Canadian Archipelago to be 32×10^9 μ-at.$\cdot$s^{-1} and a total outflow from the Arctic Ocean to be 77×10^9 μg-at.$\cdot$s^{-1} compared to an inflow of 94×10^9 μg-at.$\cdot$s^{-1} (Table 2). Using the same values for water volume transport but our values for silicate concentrations (Table 1), we find the silicate transport through the Canadian Archipelago to be 42×10^9 μg-at.$\cdot$s^{-1} and thus a total outflow from the Arctic Ocean to be 87×10^9 μg-at.$\cdot$s^{-1}, much closer to the estimated inflow and easily in agreement with it given the uncertainties involved in the water flow rates. Thus, while we do not conclude there is no removal of reactive silicate in the Arctic Ocean, with our more complete data a case for removal cannot be substantiated.

Concluding Remarks

What emerges from this work is that phosphate and silicate concentration measurements are especially useful in tracing water masses and circulation within the Canadian Archipelago and may be also useful within the Arctic Ocean surface layer. For example, our measurements in Smith Sound, assuming they are typical also of the Lincoln Sea, are consistent with the statement by Muench (1971) that Lincoln Sea water between 100 and 300 m is a mixture of Canadian and Eurasian Basin waters. Muench goes on to suggest that Canadian Basin waters dominate. Since we have no measurements from the Eurasian Basin, we cannot draw firm conclusions regarding the relative amounts of the two components, but if Greenland Sea concentrations are typical of Eurasian Basin concentrations, the opposite would be true. Based on salinity and temperature, Muench also states that water in M'Clure Strait is similar to that in the Lincoln Sea, suggesting a similar origin, and that in particular, above 150 m there seems to be a greater proportion of Canadian Basin water than is apparent in the Lincoln Sea. If, as seems likely, nutrient concentrations in M'Clure Strait resemble those in western Lancaster Sound, then the high proportion of Canadian Basin water would be consistent with our considerably higher silicate and phosphate concentrations in Lancaster and Jones sounds than in Smith Sound. The similarity of M'Clure Strait to the Lincoln Sea is not so apparent.

The clear difference in nutrient concentrations observed in the Canadian Archipelago also points out the importance of making nutrient concentration measurements in the Arctic Ocean to understand circulation and mixing there. In particular, this work indicates that the gyre in the Canadian Basin could be quite clearly defined by a series of such measurements throughout the Arctic Ocean. Also with such information it may be possible to study mixing processes, especially between the Canadian and the Eurasian Basin where it appears that fairly strong silicate and phosphate concentration gradients may exist.

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BAILEY, W. B. 1957. Oceanographic features of the Canadian Archipelago. J. Fish. Res. Board Can. 14: 731–769.

BARBER, F. G., AND A. HUYER. 1977. On the oceanography of Jones Sound, N.W.T. Manuscript Rep. Ser. 40. Marine Sciences Directorate, Department of Fisheries and Oceans, Ottawa, Canada. 48 p.

CAMPBELL, W. J. 1965. The wind driven circulation of ice and water in a polar ocean. J. Geophys. Res. 70: 3279–3301.

COACHMAN, L. K., AND K. AAGAARD. 1974. Physical oceanography of arctic and subarctic seas. *In* Yvonne Herman [ed.] Marine geology and oceanography of the arctic seas. Springer-Verlag, New York, N.Y.

CODISPOTI, L. A. 1968. Some results of an oceanographic survey in the northern Greenland Sea, summer 1964. U.S. Naval Oceanographic Office TR 202. 47 p.

CODISPOTI, L. A., AND DOROTHY LOWMAN. 1973. A reactive silicate budget for the Arctic Ocean. Limnol. Oceanogr. 18: 448–456.

CODISPOTI, L. A., AND F. A. RICHARDS. 1968. Micronutrient distributions in the East Siberian and Laptev Seas during summer, 1963. Arctic 21: 67–83.

CODISPOTI, L. A., AND T. G. OWENS. 1975. Nutrient transports through Lancaster Sound in relation to the Arctic Ocean's reactive silicate budget and the outflow of Bering Strait waters. Limnol. Oceanogr. 20: 115–119.

COLLIN, A. E. 1962. The oceanography of Lancaster Sound. Ph.D. thesis, McGill Univ., Montreal, Que.

COLLIN, A. E., AND M. J. DUNBAR. 1964. Physical oceanography in Arctic Canada. Oceanogr. Mar. Biol. (Annu. Rev.) 2: 45–75.

FROZEN SEA RESEARCH GROUP. 1977. Oceanographic data report, d'Iberville Fiord, Ellesmere Island, N.W.T. Pacific Mar. Sci. Rep. 77-23. Institute of Ocean Sciences, Patricia Bay, Sidney, B.C. 101 p.

GRASSHOFF, K. 1975. The hydrochemistry of landlocked basins and fjords. *In* J. P. Riley and G. Skirrow [ed.] Chemical oceanography. Vol. 2, 2nd ed. Academic Press, New York, N.Y. 647 p.

KINNEY, P., M. E. ARHELGER, AND D. C. BURRELL. 1970. Chemical characteristics of water masses in the Amerasian Basin of the Arctic Ocean. J. Geophys. Res. 75: 4097–4104.

MCGILL, D. A., AND N. CORWIN. 1964. Observations on the nutrient concentrations in the northern region of Baffin Bay and the Kane Basin, summer 1963. U.S. Coast Guard Oceanographic Rep. 5, CG 373-5: 37–60.

MUENCH, R. D. 1971. The physical oceanography of the northern Baffin Bay region. The Baffin Bay – North Water Project, Sci. Rep. 1. Arctic Institute of North America. 150 p.

PICKARD, G. L. 1975. Descriptive physical oceanography. 2nd ed. Pergamon Press, Inc., New York, N.Y. 214 p.

REDFIELD, A. C., B. H. KETCHUM, AND F. A. RICHARDS. 1963. The influence of organisms on the composition of sea water, p. 26–77. *In* M. N. Hill [ed.] The Sea. Vol. 2. John Wiley and Sons, New York, N.Y. 554 p.

SADLER, H. E. 1976. The flow of water and heat through Nares Strait. DREO Rep. 736. Defence Research Establishment, Ottawa. 184 p.

SIEBERT, G. H. 1968. Oceanographic observations in the Lincoln Sea, June 1967. The Baffin Bay – North Water Project Rep. 2. Arctic Institute of North America Res., Pap. 46: 22 p.

Influence of Size, Growth Rate, and Photoperiod on Cyclic Changes in Gill (Na + K)–ATPase Activity in Chinook Salmon (*Oncorhynchus tshawytscha*)

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EWING, R. D., H. J. PRIBBLE, S. L. JOHNSON, C. A. FUSTISH, J. DIAMOND, AND J. A. LICHATOWICH. 1980. Influence of size, growth rate, and photoperiod on cyclic changes in gill (Na + K)–ATPase activity in chinook salmon (*Oncorhynchus tshawytscha*). Can. J. Fish. Aquat. Sci. 37: 600–605.

Juvenile spring chinook salmon (*Oncorhynchus tshawytscha*) (Rogue River stock) were reared in constant 8°C well water under artificial lighting with photoperiods adjusted to Medford, Oregon. Juveniles were divided into six groups according to the amount of food provided. Growth (change in fork length per day) was linear from July through December for all six groups. Three distinct patterns of gill (Na + K)–ATPase activity were observed in these groups. In the two groups fed the largest rations, a peak in activity in October was observed with a smaller peak in July. Fish fed the smallest rations showed no increase in gill (Na + K)–ATPase activity throughout the year. At intermediate rations, there was a small peak in July but no peak in October. A growth rate of less than 0.027 cm/d appeared to suppress the October peak in gill (Na + K)–ATPase activity. Fish with higher growth rates showed peaks in activity in either October or November

Key words: chinook salmon, (Na + K)–ATPase, growth, photoperiod, *Oncorhynchus tshawytscha*

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Des jeunes saumons chinook (*Oncorhynchus tshawytscha*) de printemps (stock de la rivière Rogue) ont été élevés dans de l'eau de puits maintenue à une température constante de 8°C sous éclairage artificiel avec photopériodes ajustées à celles de Medford, en Orégon. Les jeunes ont été divisés en six groupes selon la quantité de nourriture offerte. Chez tous les groupes, la croissance (changement de longueur à la fourche par jour) est linéaire de juillet à décembre inclusivement. On observe chez ces groupes trois schémas distincts d'activité de la (Na + K)–ATPase branchiale. Dans les deux groupes recevant les plus grosses rations, il y a un pic d'activité en octobre et un pic moins important en juillet. Les poissons recevant les plus petites rations n'accusent aucune augmentation d'activité de la (Na + K)–ATPase branchiale tout au long de l'année. Aux rations intermédiaires, il y a un petit pic en juillet mais aucun en octobre. Un taux de croissance inférieur à 0,027 cm/d semble supprimer le pic d'octobre d'activité de la (Na + K)–ATPase branchiale. Les poissons à taux de croissance plus rapide accusent des pics d'activité soit en octobre, soit en novembre.

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PARR–SMOLT transformation in juvenile salmonids is a period of physiological adjustment to ensure a successful transition from freshwater to seawater environment (Hoar 1976). Control of parr–smolt transformation in anadromous salmonids is exerted primarily through photoperiod. Thus, silvering (Johnston and Eales 1968) and seaward migration (Wagner 1974a) are responsive to seasonal changes in photoperiod. Gills of various species show seasonal changes in (Na + K)–ATPase activity that correspond to periods of seaward migration (Zaugg and Wagner 1973; Lorz and McPherson 1976; Ewing et al. 1979). However, seawater tolerance, a manifestation of parr–smolt transformation (Hoar 1976), has been found to vary independently of photoperiod in steelhead trout (Wagner 1974b). Rapid

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adaptation to seawater appears to be size dependent (Elson 1957; Parry 1960; Wagner et al. 1969) and may be related to endogenous factors associated with the growth and/or size of the fish. These observations suggest the parr–smolt transformation requires both photoperiod and growth factors for its expression.

In an earlier report (Ewing et al. 1979) we described evidence suggesting that a minimum size of 8 cm was required in spring chinook salmon before photoperiod was effective in altering (Na + K)–ATPase activity in the gill. The present experiments were designed to examine the effects of size and growth rate on cyclic changes in gill (Na + K)–ATPase activity in spring chinook salmon.

Materials and Methods

Green eggs of spring chinook salmon from the Rogue River, Oregon, were brought to the Corvallis Research Laboratory and disinfected with an activated iodine solution (Wescodyne). The eggs were incubated in the dark in Heath incubators at 10°C until the yolk sac was completely absorbed. One thousand fry were then transferred to each of six circular fiberglass tanks, 1.5 m in diameter with 1150-L volumes. These tanks were fitted with plywood hoods and supplied with running well water at a temperature of 8 ± 1°C and a flow of ~3 L/min. A Sangamo astral timer maintained normal seasonal photoperiods for Medford, Oregon. Lighting during daylight hours was provided by four, 40-W fluorescent bulbs. Two 15-W incandescent bulbs were used to provide a 30-min twilight period each morning and evening.

All fish were fed Oregon Moist Pellet in graded sizes, according to the average weight per fish in each tank. Feeding rations were regulated by the schedules shown in Fig. 1, as modified from the R. V. Moore Company pellet feeding chart (R. V. Moore Company 1970). Between one-third and one-fourth the population of fish in each tank (250–350 fish) were weighed every 2 wk. Ration sizes were corrected for the net weight gain by use of the curves in Fig. 1. The logarithmic decline in rations as the fish size increased corrected for changes in efficiency of food conversions (R. V. Moore Company 1970). Fish were initially fed five times each day. In June, when most fish attained a length of 6 cm and were feeding vigorously, juveniles were fed slowly three times each day to maximize food consumption. Cumulative mortalities were recorded daily and feeding rations were corrected for the lost biomass.

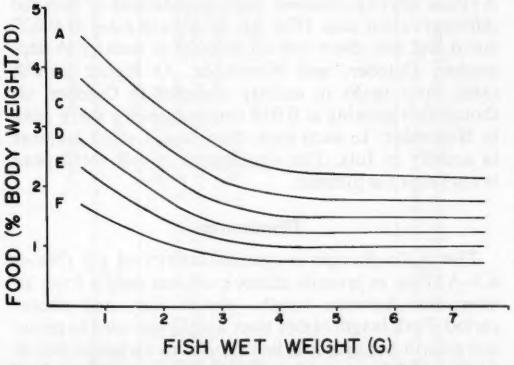

FIG. 1. Changes in food ratios with fish weight for the six feeding regimes. Final rations in % wt/d: A, 2.14%; B, 1.75%; C, 1.5%; D, 1.25%; E, 1.0%; F, 0.76%.

Enzyme Assays

Once each month, a random sample of 30 fish was taken from each of the tanks. Each fish was stunned by a blow to the head, its length and weight were measured, and the first gill arch was excised and blotted. Gill filaments were dissected away from the arch and homogenized in a medium composed of 0.2 mol/L sucrose, 0.02 mol/L Na_2EDTA, 0.10 mol/L imidazole buffer, pH 7.2, and 0.01 mol/L 2-mercaptoethanol. Analysis of (Na + K)–ATPase activity was performed according to the method of Johnson et al. (1977). Protein was measured by a modification of the method by Lowry et al. (1951).

Results

Growth Rates of Juveniles at Various Feeding Schedules

Juvenile spring chinook exhibited a linear increase in fork length for a 6-mo period (July–December, Table 1). Correlation coefficients for length in centimetres on Julian day were greater than 0.95 in all groups. Growth rates increased linearly with ration size expressed as percentage of body weight per day (Fig. 2). At consumption rates of 1.75% body weight/d and above, only a slight increase in growth rate occurred, indicating that maximum conversion of food had been reached. Ad libitum feeding of the same stock of fish under similar conditions in 1975 resulted in a similar growth rate. Maintenance rations, the rations at which neither growth nor weight loss occurred, were not determined in this experiment.

Changes in Gill (Na + K)–ATPase Activity with Feeding Regime

Gill (Na + K)–ATPase activity showed three patterns related to growth rates (Fig. 3). In the two groups

TABLE 1. Growth rates of spring chinook at 8°C from July to December.

Final feeding regime (% body wt/d)	Regression equation length on calendar day	r^2
0.76	4.011+0.0129×	0.96
1.00	3.968+0.0166×	0.96
1.25	2.862+0.0233×	0.99
1.50	2.914+0.0259×	0.99
1.75	1.104+0.0342×	0.997
2.15	1.235+0.0338×	0.99

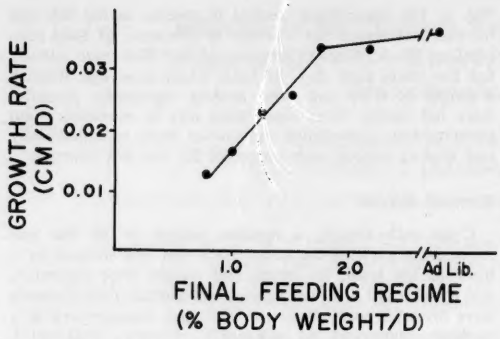

FIG. 2. Growth rates (cm/d) of juvenile spring chinook salmon at various feeding regimes. As fish grew, rations were changed as in Fig. 1 to ensure linear growth. Ad libitum feeding of the same stock of fish under similar conditions was performed in 1975.

with highest rations and at growth rates greater than 0.026 cm/d, gill (Na + K)–ATPase activity peaked in October, with a shoulder in July. This pattern was similar to that reported earlier with ad libitum feeding at the same temperature (Ewing et al. 1979). In the three groups with intermediate rations, and growth rates between 0.016 and 0.026 cm/d, activity did not peak in October, although there was a peak in activity in July. In the group with the smallest rations, growth rate was only 0.013 cm/d, and no peak in activity occurred. Gill (Na + K)–ATPase activity declined throughout the season.

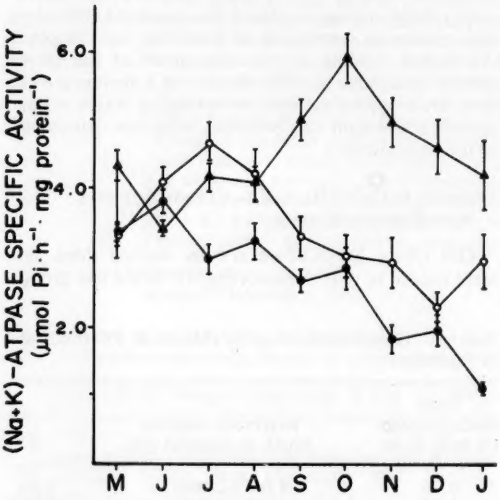

FIG. 3. Changes in gill (Na + K)–ATPase activity with time at three different feeding regimes. Values represent means ± standard errors for 30 fish samples. Final rations: 0.76% body wt/d (●); 1.5% body wt/d (○); 2.15% body wt/d (▲).

EFFECTS OF SIZE ON GILL (Na + K)–ATPASE ACTIVITY

In experiments utilizing suboptimal food rations, there are inequities in the food distribution related to the aggressiveness of individual fish. Thus, in the experiments described above, growth rates under each feeding regime (Table 1) were averages for large populations of fish growing at different individual rates. These different growth rates resulted in increased ranges in fork length. To look at the changes in gill (Na + K)–ATPase activity in fish growing within narrow ranges of growth rate, it was necessary to determine the relationship between fork length and to use these data to simulate changes in gill (Na + K)–ATPase activity in fish growing at particular growth rates.

To accomplish this, data from all experimental tanks were pooled for each month and sorted into groups of 1-cm increments. Pooled data were used to increase the number of samples in each size category. There was a complex relationship between fork length, time of year, and gill (Na + K)–ATPase activity (Table 2). At any month, there was a positive relationship between fork length and gill (Na + K)–ATPase activity (Fig. 4). However, for fish of a given length, the specific activity decreased with time.

From the linear growth rates between July and December (Table 1) and the average fork length of 7.16 ± 0.60 (SE, $n = 179$) in July, we calculated the minimum growth rates required to attain different length classes by October (Table 3). By interpolation from Table 2, it was possible to simulate changes in gill (Na + K)–ATPase activity for fish growing at these different growth rates throughout the season. The result (Fig. 5) resembled the pattern of gill (Na + K)–ATPase activity obtained from populations of fish fed different ration sizes (Fig. 3). At growth rates of 0.027 cm/d and less, there was no increase in activity in September, October, and November. At higher growth rates, most peaks in activity occurred in October, although fish growing at 0.038 cm/d showed a sharp peak in November. In each case, there was a small increase in activity in July. The significance of this small peak is not known at present.

Discussion

The cyclic changes in specific activity of gill (Na + K)–ATPase in juvenile chinook salmon results from an interaction between length, growth rate, and photoperiod. Fork length rather than weight was used to measure growth because it is less subject to variation due to feeding and less sensitive to changes in the physiology of fish during parr–smolt transformation (Vanstone and Markert 1968; Fessler and Wagner 1969).

TABLE 2. Seasonal variation of gill (Na + K)-ATPase activities in juvenile chinook of various size-classes.[a]

Size (cm)	May	June	July	August	September	October	November	December	January
4.0–4.9	2.77 (1)	2.80 ± 0.09 (3)							
5.0–5.9	3.17 ± 0.09 (59)	3.26 ± 0.10 (59)	2.49 ± 0.27 (14)	2.27 ± 0.20 (8)	2.31 ± 0.32 (3)				
6.0–6.9	3.63 ± 0.10 (57)	4.05 ± 0.09 (88)	3.75 ± 0.14 (60)	2.92 ± 0.19 (30)	2.29 ± 0.18 (16)	2.54 ± 0.26 (11)	0.77 ± 0.20 (5)	1.82 ± 0.33 (7)	
7.0–7.9	5.29 ± 1.30 (2)	4.49 ± 0.36 (29)	4.76 ± 0.13 (81)	3.64 ± 0.15 (56)	2.82 ± 0.24 (34)	3.16 ± 0.26 (19)	1.42 ± 0.16 (15)	1.62 ± 0.14 (14)	1.10 ± 0.10 (8)
8.0–8.9		6.53 (1)	5.14 ± 0.21 (21)	4.66 ± 0.16 (53)	4.09 ± 0.16 (48)	3.02 ± 0.22 (40)	1.64 ± 0.12 (30)	1.59 ± 0.14 (19)	0.97 ± 0.09 (10)
9.0–9.9			5.26 ± 0.31 (3)	4.96 ± 0.20 (29)	4.91 ± 0.22 (41)	3.97 ± 0.36 (36)	2.35 ± 0.20 (36)	2.04 ± 0.14 (36)	1.77 ± 0.30 (19)
10.0–10.9				5.88 ± 0.89 (2)	6.43 ± 0.22 (28)	6.34 ± 0.46 (23)	3.19 ± 0.23 (32)	2.66 ± 0.25 (32)	1.47 ± 0.11 (20)
11.0–11.9					7.72 ± 0.48 (7)	7.73 ± 0.43 (26)	5.36 ± 0.42 (24)	2.51 ± 0.37 (10)	1.65 ± 0.16 (16)
12.0–12.9					5.08 ± 0.47 (7)	7.48 ± 0.65 (7)	6.43 ± 0.37 (16)	4.20 ± 0.32 (18)	2.69 ± 0.54 (15)
13.0–13.9						5.10 ± 0.13 (4)	8.41 ± 0.63 (9)	5.54 ± 0.57 (8)	3.88 ± 0.45 (7)
14.0–14.9							7.00 ± 0.39 (7)	6.72 ± 0.34 (22)	4.51 ± 0.72 (10)
15.0–15.9							7.67 ± 1.44 (3)	6.97 ± 0.33 (12)	4.29 ± 0.42 (9)

[a]Values are means ± standard errors. Number of samples in each size-class are given in parentheses. Data was obtained from pooled samples from the six feeding regimes sorted into 1.0-cm-size-classes by computer.

TABLE 3. Growth rates of juvenile chinook salmon necessary to attain given length classes by October, assuming that fish are a mean size of 7.16 cm in July and that growth rates are linear from July to October.

Length (cm)	Growth rate (cm/d)
13.0–13.9	0.071
12.0–12.9	0.060
11.0–11.9	0.049
10.0–10.9	0.038
9.0–9.9	0.027
8.0–8.9	0.016

It is not possible in the present set of experiments to separate effects due to changes in growth rate from those resulting from attaining a given fork length at a particular time. Because growth and length are highly correlated, we cannot determine whether the ATPase cycle was suppressed at lowest feeding rations (Fig. 3) because the fish had not attained 8.0 cm by September, as proposed earlier (Ewing et al. 1979), or because growth rate was less than 0.026 cm/d.

The influence of size and/or growth rate on gill (Na + K)-ATPase activity is not unexpected, as similar phenomena have been observed in studies of osmoregulation in chinook salmon. Wagner et al. (1969) showed that growth rate influenced the osmoregulatory capabilities of juvenile chinook salmon introduced into seawater. They suggested that this might result either from a more functional regulatory system or an increased capability to initiate the regulatory system when introduced into seawater. Our present results show that larger fish have greater

FIG. 4. Gill (Na + K)-ATPase specific activities of fish of various size-classes by month. Values represent means ± standard errors of variable numbers of fish. May (●); July (○); September (△); November (▲); January (□).

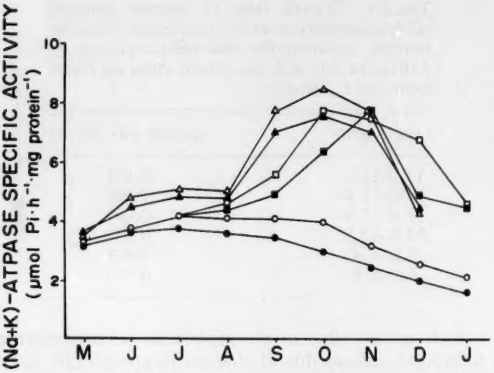

FIG. 5. Gill (Na + K)–ATPase specific activities of fish with hypothetical growth rates. Specific activity data were derived from interpolation of data in Table 2. Growth rates: 0.016 cm/d (●); 0.027 cm/d (○); 0.038 cm/d (■); 0.049 cm/d (□); 0.060 cm/d (▲); 0.071 cm/d (△).

(Na + K)–ATPase activities and therefore at least a portion of the osmoregulatory system is more functional at the larger sizes. Wagner et al. (1969) further suggest that increased capacity to initiate osmoregulatory systems may also result from increased size as well as growth rates. Thus, both the capacity to osmoregulate and ability to respond to seawater challenges may be modified by size and/or growth.

Similar observations have been made in other species. Parry (1960) found that size and growth rate were important in salinity tolerance in *Salmo salar*. She suggested that size and age of the fish might affect the content of "Keys–Willmer cells" in the gill with a subsequent increase in osmoregulatory capacity. Other observations on *S. salar* (Elson 1957; Houston 1961; Knutsson and Grav 1976) have confirmed these results. The increase in gill (Na + K)–ATPase activity that we have observed is consistent with this hypothesis, because most of the (Na + K)–ATPase activity in the gill is associated with chloride (Keys–Willmer) cells (Kamiya 1972; Sargent et al. 1975).

A number of size thresholds for seawater adaptation and parr–smolt transformation have been described. Wagner (1974b) found that seawater tolerance of juvenile steelhead trout (*Salmo gairdneri*) was fully developed when fish exceeded 12–13 cm. Similarly, in a study of parr–smolt transformation in Atlantic salmon, Farmer et al. (1978) found that little change in lipid content or condition factor occurred in juvenile Atlantic salmon less than 12 cm. Exposure of parr to seawater resulted in mortalities only in fish less than 12 cm. Knutsson and Grav (1976) found that seawater tolerance of Atlantic salmon was developed by 11–14 cm. Mahnkin (1973) found that introduction of juvenile coho salmon (*Oncorhynchus kisutch*) into seawater resulted in large numbers of mortalities and reversions to a parr-like state in fish less than 15 g (~12 cm). In chinook salmon, tolerance to seawater developed by 12–15 cm (Wagner et al. 1969). These thresholds may reflect either changes in physiological processes permitting better survival or decreased surface area/volume ratios which would lessen the effects of the osmotic imbalances imposed on the fishes. It is not possible to assign the relative importances of these factors at the present time.

Acknowledgments

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ELSON, P. F. 1957. The importance of size in the change from parr to smolt in Atlantic salmon. Can. Fish. Cult. 21: 1–6.

EWING, R. D., S. L. JOHNSON, H. J. PRIBBLE, AND J. A. LICHATOWICH. 1979. Environmental effects of cyclic changes in gill (Na + K)–ATPase activity in chinook salmon, *Oncorhynchus tshawytscha*. J. Fish. Res. Board Can. 36: 1347–1353.

FARMER, G. J., J. A. RITTER, AND D. ASHFIELD. 1978. Seawater adaptation and parr–smolt transformation of juvenile Atlantic salmon, *Salmo salar*. J. Fish. Res. Board Can. 35: 93–100.

FESSLER, J. L., AND H. H. WAGNER. 1969. Some morphological and biochemical changes in steelhead trout during the parr–smolt transformation. J. Fish. Res. Board Can. 26: 2823–2934.

HOAR, W. S. 1976. Smolt transformation: evolution, behavior and physiology. J. Fish. Res. Board Can. 33: 1233–1252.

HOUSTON, A. H. 1961. Influence of size upon adaptation of steelhead trout (*Salmo gairdneri*) and chum salmon (*Oncorhynchus keta*) to seawater. J. Fish. Res. Board Can. 18: 401–415.

JOHNSON, S. L., R. D. EWING, AND J. A. LICHATOWICH. 1977. Characterization of gill (Na + K) – activated adenosine triphosphatase from chinook salmon, *Oncorhynchus tshawytscha*. J. Exp. Zool. 199: 345–354.

JOHNSTON, C. E., AND J. G. EALES. 1968. Influence of temperature and photoperiod on guanine and hypoxanthine levels in skin and scales of Atlantic salmon (*Salmo salar*) during parr–smolt transformation. J. Fish. Res. Board Can. 25: 1901–1909.

KAMIYA, M. 1972. Sodium–potassium-activated adenosine triphosphatase in isolated chloride cells from eel gills. Comp. Biochem. Physiol. 43B: 611–617.

KNUTSSON, S., AND T. GRAV. 1976. Seawater adaptation in Atlantic salmon (*Salmo salar* L.) at different experimental temperatures and photoperiods. Aquaculture 8: 169–187.

LORZ, H. W., AND B. P. MCPHERSON. 1976. Effects of copper or zinc in fresh water on the adaptation of sea water and ATPase activity, and the effects of copper on migratory disposition of coho salmon (*Oncorhynchus kisutch*). J. Fish. Res. Board Can. 33: 2023–2030.

LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, AND R. J. RANDALL. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 192: 265–275.

MAHNKIN, C. V. W. 1973. The size of coho salmon and time of entry into seawater. Part 1. Effects of growth and condition index. 24th N.W. Fish. Cultural Conference Abstract.

MOORE, R. V., COMPANY, INC. 1970. Oregon Pellet Feeding Chart. LaConner, Washington, USA.

PARRY, G. 1960. The development of salinity tolerance in the salmon, *Salmo salar* (L.) and some related species. J. Exp. Biol. 37: 425–434.

SARGENT, J. R., A. J. THOMSON, AND M. BORNANCIN. 1975. Activities and localization of succinic dehydrogenase and Na^+/K^+-activated adenosine triphosphatase in the gills of freshwater and seawater eels (*Anguilla anguilla*). Comp. Biochem. Physiol. 51B: 75–79.

VANSTONE, W. E., AND J. R. MARKERT. 1968. Some morphological and biochemical changes in coho salmon, *Oncorhynchus kisutch*, during parr–smolt transformation. J. Fish. Res. Board Can. 25: 2403–2418.

WAGNER, H. H. 1974a. Photoperiod and temperature regulation of smolting in steelhead trout (*Salmo gairdneri*). Can. J. Zool. 42: 219–234.

1974b. Seawater adaptation independent of photoperiod in steelhead trout (*Salmo gairdneri*). Can. J. Zool. 52: 805–812.

WAGNER, H. H., F. P. CONTE, AND J. L. FESSLER. 1969. Development of osmotic and ionic regulation in two races of chinook salmon, *Oncorhynchus tshawytscha*. Comp. Biochem. Physiol. 29: 325–341.

ZAUGG, W. S., AND H. H. WAGNER. 1973. Gill ATPase activity related to parr–smolt transformation and migration in steelhead trout (*Salmo gairdneri*). Influence of photoperiod and temperature. Comp. Biochem. Physiol. 45B: 955–965.

Bongo Net Retention Rates as Effected by Towing Speed and Mesh Size

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COLTON, J. B. JR., J. R. GREEN, R. R. BYRON, AND J. L. FRISELLA. 1980. Bongo net retention rates as effected by towing speed and mesh size. Can. J. Fish. Aquat. Sci. 37: 606–623.

A comparison of 0.253- and 0.333-mm mesh bongo net retention rates at towing speeds of 1.5 and 3.5 knots showed no significant mesh size related variation in filtration efficiency or in abundance of larval herring at either towing speed. For 1.5-knot tows, there was no significant mesh size difference in larval herring length frequency distribution. For 3.5-knot tows, the distributions of lengths were significantly different for the two mesh sizes. The condition of herring larvae was noticeably better in the 1.5-knot tow samples (3% of yolk-sac larvae caught in 3.5-knot tows had their yolk sac intact compared to 50% from 1.5-knot tows). Feeding incidence was higher for larvae caught in 1.5-knot tows (71%) than for larvae caught in 3.5-knot tows (50%).

At a towing speed of 3.5 knots the 0.253-mm mesh zooplankton displacement volumes were significantly greater than the 0.333-mm mesh displacement volumes. At a towing speed of 1.5 knots the difference in displacement volumes was not significant. Adult *Oithona* spp. and *Pseudo-Paracalanus* spp., unidentified copepodites, and adult and juvenile *Hyperia* spp. were caught in significantly greater numbers by the 0.253-mm mesh net at both towing speeds. The 0.253-mm/0.333-mm abundance ratios were greatest for the smaller and more abundant invertebrate taxa. The variation in zooplankton retention rates resulted in large part from differences in mesh size, although catching efficiency was also influenced by tow speed. A substantial proportion of the food items in the guts of all size groups of larval herring consisted of taxa which were not retained by either mesh net (invertebrate eggs, copepod nauplii, *Oithona* spp., and *Pseudo-Paracalanus* spp. copepodites). Size was the most important factor in prey selection and the average size of food items increased with increased length of herring larvae. On the basis of this analysis, sampling strategies are suggested for future surveys.

Key words: bongo nets, retention rates, ichthyoplankton, larval herring, zooplankton, feeding incidence

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Une comparaison des taux de rétention de filets «bongo» à mailles de 0,253 mm et 0,333 mm traînés à des vitesses de 1,5 et 3,5 noeuds n'indique pas de variation significative liée à la grandeur des mailles dans l'efficacité de filtration ou abondance des larves de hareng à l'une ou l'autre vitesse de traînée. Dans le cas de traits à 1,5 noeud, il n'y a pas de différence de distribution des fréquences de longueur des larves de hareng en fonction de la grandeur des mailles. Dans le cas de traits à 3,5 noeuds, il y a différences marquées de distribution des longueurs entre les deux grandeurs de mailles. Les échantillons prélevés à 1,5 noeud contiennent des larves de hareng dont la condition est notablement meilleure (3% des alevins vésiculés capturés à 3,5 noeuds ont un sac vitellin intact comparativement à 50% dans les traits à 1,5 noeud). Les larves capturées à 1,5 noeud offrent une fréquence d'alimentation plus élevée (71%) que celles capturées dans les traits à 3,5 noeuds (50%).

A une vitesse de traînée de 3,5 noeuds, les volumes de déplacement du zooplancton avec mailles de 0,253 mm sont nettement plus grands qu'avec mailles de 0,333 mm. A une vitesse de 1,5 noeud, la différence de volumes de déplacement n'est pas significative. Des *Oithona* spp. et *Pseudo-Paracalanus* spp. adultes, copépodites non identifiés et *Hyperia* spp. adultes et jeunes sont capturés en nombres nettement plus grands par le filet de mailles de 0,253 mm aux deux vitesses de traînée. Les rapports d'abondance de 0,253 mm/0,333 mm ont des valeurs maximales chez les taxons d'invertébrés petits et abondants. La variation des taux de rétention du

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[2]It was originally planned to sample alongside a buoy attached to a parachute drogue set at mid-sampling depth in an effort to remain in a specific water mass and population of larvae, but gear problems and time restraints forced us to abandon this sampling scheme.

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zooplancton résulte en grande partie de différences de grandeur de mailles, bien que l'efficacité de capture soit aussi influencée par la vitesse de traînée. Une proportion substantielle des organismes trouvés dans le tractus digestif des larves de hareng de toutes tailles consiste en taxons qui ne sont pas retenus par l'une ou l'autre grandeur de mailles (oeufs d'invertébrés, nauplii de copépodes, *Oithona* spp. et copépodites de *Pseudo-Paracalanus*). La taille n'est pas un facteur important dans le choix des proies et la taille moyenne de ces dernières augmente avec la longueur des larves de hareng. A partir de cette analyse, nous suggérons des stratégies d'échantillonnage pour relevés futurs.

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SEVERAL investigators have considered the effectiveness of towed nets in sampling the plankton community. However, few quantitative studies have been made on mesh retention of zooplankton in general and fish larvae and their zooplankton prey in particular. A thorough theoretical discussion of filtration performance considering net shape, mesh size, tow speed, and clogging rates is given by Tranter and Smith (1968). Laboratory and field studies by Saville (1958) and field observations by Smith et al. (1968) indicate that the maximum cross-sectional diameter of an organism must be greater than the mesh diagonal if it is to be fully retained. Lenarz (1972) concluded, however, that the mesh "diagonal rule" was too conservative for the case of northern anchovy (*Engraulis mordax*) larvae sampled with slowly towed (1–1¼ knots) nylon nets. The degree of extrusion is strongly dependent on filtration velocity and also contingent on body shape (Vannucci 1968), so that the loss of fusiform larval fish species is less than that of filiform species.

We undertook this study to examine the magnitude of possible biases arising from the use of standard sampling gear and methods in routine ichthyoplankton surveys. In particular, we have looked for biases resulting from differential retention of fish larvae of varying lengths and the effects of mesh size and tow speed on our assessment of larval feeding and the structure of the invertebrate zooplankton community.

An initial comparison of the abundance of larval herring from 113, 3.5-knot, paired 0.505- and 0.333-mm mesh bongo net (Smith and Richardson 1977) samples obtained on *Albatross IV* Cruise 73-9 (December 4–20, 1973) indicated that: (1) 4–6-mm herring were not fully retained by either mesh; (2) 7–12-mm larvae were more fully retained by the 0.333-mm mesh; and (3) retention of larvae larger than 12 mm was similar for both mesh sizes (Fig. 1, Table 1). Such survey data, however, are not amenable to this type of comparison for the following reasons: the samples were collected over a wide area and considerable time period, contagious distribution of larvae, populations of varying age structures, high percentage of negative tows. A

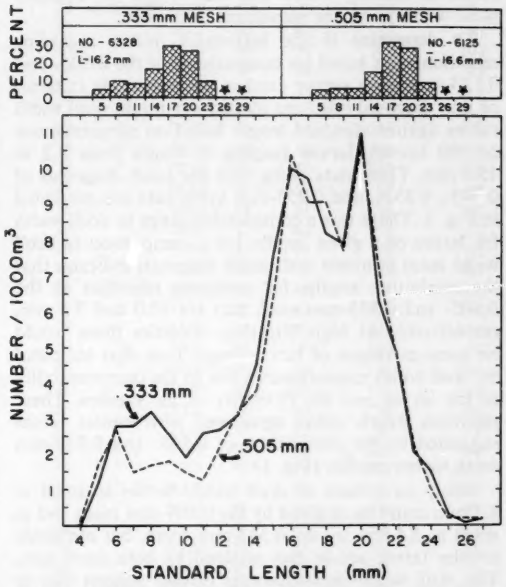

FIG. 1. Total catch, mean length, length frequency (3-mm size groups), and average abundance (1-mm size groups) of larval herring for 0.333- and 0.505-mm mesh nets, *Albatross IV* Cruise 73-9 (★ = less than one).

TABLE 1. Average abundance (no./1000 m³) of larval herring for 0.505- and 0.333-mm mesh nets and 0.333-mm mesh/0.505-mm mesh abundance ratios, *Albatross IV* Cruise 73-9.

	Length (mm)								
	4–6	7–9	10–12	13–15	16–18	19–21	22–24	25–27	Total
0.505-mm mesh	4.49	5.06	5.13	13.46	28.61	25.63	8.06	0.52	91.38
0.333-mm mesh	3.95	8.62	7.25	15.17	27.81	25.25	8.53	0.54	97.22
0.333/0.505 ratio	0.88	1.70	1.41	1.13	0.97	0.99	1.06	1.04	1.06

t-test of logarithmically transformed data for positive tows showed that the differences in average abundance values for the 0.505- and 0.333-mm mesh nets were not significant for any length group of larvae.

In studies of mesh retention of larval fishes, body depth at the insertion of the pectoral fin has frequently been used as the critical dimension to compare with the mesh diagonal. However, Wood (1972) suggested that for herring larvae maximum skull width is a more meaningful measurement as this is the first part of the body to ossify and thus less easily compressed or crushed. We have measured maximum skull widths (excluding eyeballs) and body depths at the base of the pectorals of 200 herring larvae ranging in length from 4.2 to 9.9 mm. The average body depth (excluding yolk sac, if present) was 0.32 mm and the average skull width was 0.50 mm. With respect to mesh retention of larval herring within this size range, we consider skull width to be the significant dimension.

To determine if the inferences drawn regarding retention rates based on comparisons of the 0.505- and 0.333-mm mesh survey catches were plausible in terms of the "diagonal rule," we plotted maximum skull width values against standard length based on measurements of 300 herring larvae ranging in length from 4.2 to 15.1 mm. These data along with the mesh diagonals of 0.505-, 0.333-, and 0.253-mm nylon nets are presented in Fig. 2. There was a considerable range in skull width for larvae of a given length, but a comparison of skull width measurements with mesh diagonals indicates that the minimum lengths for complete retention in the 0.505- and 0.333-mm mesh nets are 12.0 and 7.5 mm, respectively. At high filtration velocities there would be some extrusion of larvae larger than that indicated by skull width measurements due to the compressibility of the larvae and the flexibility of the meshes. These retention length values agree well with similar values suggested by the comparison of 0.505- and 0.333-mm mesh survey catches (Fig. 1).

Solely on a basis of skull width, larvae as small as 8.5 mm could be retained by the 0.505-mm mesh and as small as 5.5 mm by the 0.333-mm mesh, but obviously smaller larvae are in fact retained by both mesh nets. The skull width measurements further suggest that to ensure full retention of larvae smaller than 7 mm, it would be necessary to use netting having a mesh diagonal of 0.36 mm or less (mesh aperture of 0.253 mm or less).

We concluded from this preliminary examination of survey data that an assessment of the limitations imposed by mesh selectivity on adequate estimates of plankton populations would require special field studies designed to circumvent those previously listed problems associated with the use of survey data. In this paper we summarize results of an initial field study made in an effort to obtain a more precise measure of the effects of tow speed and mesh aperture on the size-specific retention rates of larval herring and associated invertebrate forage organisms.

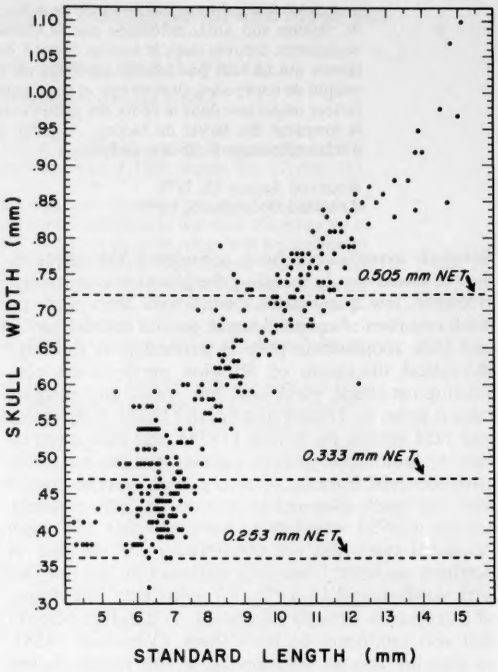

FIG. 2. Relationship between standard length and maximum skull width for larval herring. The horizontal broken lines indicate the mesh diagonals of 0.505-, 0.333-, and 0.253-mm nets.

Materials and Methods

Two 48-h stations were occupied from November 1–5, 1975 (*Albatross IV* Cruise 75-13) in the Great South Channel that separates Nantucket Shoals and Georges Bank. Two series of double oblique tows were made within a 2-mi² (2.6 km²) area centered at 41°12′N latitude and 69°18′W longitude using paired 61-cm bongo samplers fitted with 0.333-mm (port) and 0.253-mm (starboard) nylon mesh nets.[2] At the first station, 43 tows were made at 3.5 knots (6.5 km/h); at the second station, 47 tows at 1.5 knots (2.8 km/h). New nets were used for each series of tows. The sampler accessories (flow meter, time–depth recorder, towing wire, and wire depressor) were rigged and of specifications similar to those described by Smith and Richardson (1977) with the exception that plastic codend buckets were used in an effort to minimize damage to the fish larvae. The gear was deployed at 50 m/min and retrieved at 10 m/min. The basic station data are given in Table 2.

Fish larvae were sorted from the 0.333-mm and 0.253-mm mesh samples and enumerated by species. For species numbering less than 100, all larvae were measured for standard length to the nearest 0.1 mm. In samples containing greater numbers of larvae, a subsample of at least 100 specimens was taken for length determinations and the total length frequency determined by multiplying the

TABLE 2. Date, time, depth, volume-filtered summaries for 3.5- and 1.5-knot tows, *Albatross IV* Cruise 75-13.

3.5-knot station (43 tows)							1.5-knot station (47 tows)						
					Vol. filtered (m^3)							Vol. filtered (m^3)	
Tow No.	Date	Local time	Water depth (m)	Sample depth (m)	0.333-mm mesh	0.253-mm mesh	Tow No.	Date	Local time	Water depth (m)	Sample depth (m)	0.333-mm mesh	0.253-mm mesh
1	1/XI/75	0806	53	51	298	301	1	3/XI/75	1300	49	45	128	130
2	"	0859	53	43	263	261	2	"	1359	42	40	146	147
3	"	1100	51	50	293	292	3	"	1500	50	50	122	124
5	"	1303	49	40	319	305	5	"	1700	53	42	167	170
6	"	1405	46		307	314	6	"	1804	53	41	130	129
7	"	1910	46		243	241	7	"	1900	49	40	89	89
8	"	2001	51		217	220	8	"	2002	51	43	70	69
10	"	2158	60	47	200	193	9	"	2121	51	43	83	112
11	"	2255	57	57	227	228	10	"	2200	55	47	113	112
13	2/XI/75	0103	49	40	278	277	11	"	2258	55	49	127	126
14	"	0203	49	38	236	236	12	4/XI/75	0003	55	39	95	95
15	"	0300	48	42	237	234	13	"	0102	60	41	87	87
16	"	0400		39	204	205	14	"	0159	49	39	103	104
17	"	0500	51	50	188	189	15	"	0258	49	38	90	84
18	"	0600	46	44	151	149	16	"	0357	46	38	85	85
19	"	0700	53	49	205	207	17	"	0502	49	40	113	113
20	"	0804	49	42	202	204	18	"	0600	49	34	137	137
21	"	0858	48	41	231	233	19	"	0658	46	40	103	104
22	"	0956	51	41	237	241	20	"	0758	49	40	102	102
23	"	1053	49	38	297	290	21	"	0859	58	52	69	67
24	"	1205	51	38	242	237	22	"	1002	58	51	91	91
25	"	1300	49	36	272	266	23	"	1056	53	42	93	92
26	"	1416	53	32	287	281	24	"	1202	53	43	49	47
27	"	1502	46	30	303	299	25	"	1259	48	45	85	85
28	"	1608	46	38	242	240	26	"	1359	48	48	101	101
30	"	1805	53	45	211	213	27	"	1458	48	42	109	109
31	"	1900	46	44	187	188	28	"	1559	46	40	122	120
32	"	2000	51	38	248	251	29	"	1700	48	40	100	99
33	"	2057	64	41	227	228	30	"	1801	46	44	111	108
34	"	2159	70	47	267	267	31	"	1900	48	40	116	115
35	"	2257	49	47	295	296	32	"	2001	48	38	98	96
36	3/XI/75	0004	51	39	279	276	33	"	2100	57	53	57	52
37	"	0104	53	37	301	304	34	"	2202	70	53	103	99
38	"	0202	53	35	278	270	35	"	2301	57	55	125	123
39	"	0300	71	35	271	267	36	5/XI/75	0001	57	39	103	102
40	"	0402	49	38	259	262	37	"	0102	58	40	95	95
41	"	0500	48	42	211	213	38	"	0203	55	55	85	83
42	"	0602	49	41	283	285	39	"	0259	53	43	90	86
44	"	0800	51	48	199	190	40	"	0401	48	43	95	91
45	"	0855	57	50	228	242	41	"	0503	49	41	105	101
46	"	0958	60	48	237	237	42	"	0602	57	45	165	161
47	"	1059	58	47	261	262	43	"	0703	53	37	175	172
48	"	1203	49	38	269	263	44	"	0800	49	42	184	179
							45	"	0858	48	48	152	150
							46	"	0959	57	43	129	126
							47	"	1057	58	43	138	137
							48	"	1203	48		51	48
Avg.			52[a]	42[a]	249	248				52[a]	43[a]	108	108

[a]For tows in which both water depth and tow depth values obtained.

number at each length interval by the reciprocal of the aliquot fraction. The flow-meter readings for each tow were converted to m^3 of water filtered and larval abundances expressed in terms of numbers/1000 m^3 water filtered. Thirteen species of larval fishes were collected, but only Atlantic herring larvae occurred in all tows and over a size range amenable to an analysis of retention rates.

After removal of larval fishes, invertebrate plankton displacement volumes were measured by the method described by Ahlstrom and Thrailkill (1963) with the exception that

the sample was allowed to drain until the interval between drops from the bottom of the draining cone was 15 s. Displacement volumes were expressed in terms of cm³/1000 m³ water filtered.

The 0.333- and 0.253-mm mesh samples from 10 randomly selected 3.5- and 1.5-knot tows were then aliquoted with a Folsom splitter (McEwan et al. 1954) to ~500 zooplankters. All organisms within this subsample were sorted and major groups (Copepoda, Chaetognatha, Cladocera, Amphipoda, Euphausiacea, and Annelida) enumerated by species or lowest taxon possible. Length measurements were made on 20 randomly selected individuals of each taxon according to methods described by Isaacs et al. (1969). Abundances were determined by multiplying the number enumerated by the reciprocal of the aliquot fractions and expressed in terms of numbers/m³ water filtered.

Finally, the gut contents were identified and enumerated for a random sample of 20 larval herring from each of five 0.333- and 0.253-mm mesh, 3.5- and 1.5-knot daytime tows used for zooplankton abundance estimates. Each larva examined was measured to the nearest 0.1 mm and the alimentary tract dissected out and cleared and fixed for 12–24 h in dilute lactic acid – methyl blue solution. The gut was dissected and examined under 25–50× magnification. Prey organisms were identified to lowest taxa possible.

Results and Discussion

Filtration Efficiency

As indicated by the marked similarity of the volume-filtered values (Table 2), a *t*-test (Snedecor and Cochran 1967) showed no significant variations in the filtration efficiencies at either towing speed due to differences in the porosity of the 0.253- and 0.333-mm mesh nets. The ratio of the volumes of water filtered at 3.5 and 1.5 knots was proportional to the towing speed ratio (2.3/1).

Size Composition of Larval Herring

Length frequencies and mean lengths of larval herring retained in both sizes of nets for individual and total 3.5- and 1.5-knot tows are presented in Tables 3 and 4. The percentage length frequencies by 3-mm-

Table 3. Length frequencies and mean lengths of larval herring for 0.253- and 0.333-mm mesh nets, 3.5-knot station.

	Length (mm)	Tow number																						
		1	2	3	5	6	7	8	10	11	13	14	15	16	17	18	19	20	21	22	23	24	25	26
0.253-mm mesh	4																							
	5		4					3										1						
	6	4	23	19	4	12	7	8	4	5		1	4	1			7	3	3	3	18		4	
	7	38	59	73	30	44	42	30	50	34	36	14	40	21	21	24	33	29	26	42	95	53	28	4
	8	139	101	97	35	76	80	72	65	72	104	34	58	37	24	48	110	23	15	66	70	65	49	30
	9	105	51	97	53	37	73	74	94	62	64	10	26	17	38	50	67	10	4	22	55	53	44	32
	10	63	43	50	48	25	91	39	104	52	123	26	42	28	55	43	27	5	2	6	5	61	42	57
	11	55	31	19	24	18	28	22	32	31	53	11	24	29	26	13	17			4	5	18	24	45
	12	12	20	8	30	4	11	11	4		8	2	6	5	9	6	3	1		2	2	8	6	9
	13	4	16	19	11	4	10	8			4	1					3						2	
	14		31	4	16	4	7	5	4		4											3		2
	15		4		11	4			4							2								
	16				3			3														3		
	17									1										2				
	18				3																			
	19	4																		2				
	20																							
	21	4																						
	Total	420	391	386	268	228	349	275	361	257	396	99	200	138	173	186	334	72	50	149	250	264	199	179
	Mean	9.4	9.0	8.8	10.2	8.7	9.2	9.1	9.1	8.9	8.8	8.9	8.8	9.1	9.4	9.0	9.0	7.8	7.5	8.3	7.8	8.9	9.0	9.8
0.333-mm mesh	4																			2				
	5						17														5	5		
	6	10	4	4	8	5	21		6	15	9	2	4		2			7	3	10	7	2		
	7	80	32	65	47	38	82	8	38	40	48	47	21	17	26	23	38	29	19	72	59	37	4	5
	8	142	72	115	65	53	79	78	84	68	50	46	26	20	28	31	97	32	25	52	100	53	19	18
	9	101	77	65	29	53	79	58	61	48	51	48	21	24	39	79	94	8	11	26	40	49	35	30
	10	80	63	58	57	58	67	59	75	28	86	38	36	35	47	48	89	1	1	10	21	46	59	66
	11	32	50	29	18	10	33	27	14	9	42	13	12	18	19	21	35				4	16	53	26
	12	37	36	7	18	15	21	16	6	8	6	8	2	3	9	2	19	1				12	23	5
	13	16	40	7	8	3	9	8		2	6			1		4	7			2		2	5	
	14	16	32	7	8	15		6				2			2							5	4	2
	15	5	18		3	3	4	6	5			4			2		6	1				5	2	
	16	4	22	4				6							2			1						3
	17		4																					2
	18																							
	19																							
	20	3																						
	21						1																	
	Total	526	450	361	261	253	413	272	289	218	298	208	122	118	176	208	385	80	59	174	236	232	204	157
	Mean	9.3	10.6	8.9	9.2	9.3	8.7	9.8	8.9	8.5	9.1	8.9	8.9	9.3	9.4	9.2	9.3	7.8	7.8	7.8	8.0	9.1	10.3	10.0

TABLE 3 (*Concluded*)

	Length (mm)	Tow number																			Total	
		27	28	30	31	32	33	34	35	36	37	38	39	40	41	42	44	45	46	47	48	No.	%
0.253-mm mesh	4																						
	5									8							4		6	2		28	2.9
	6			2	4	22		4	5	28	3			1		1	5	9	30	3	23	270	
	7	8	3	20	37	58	12	24	109	39	59	7	4	3	7	18	25	23	53	3	32	1410	
	8	10	10	58	40	94	165	119	164	165	81	14	6	7	3	9	57	68	45	5	49	2639	61.1
	9	5	1	15	31	98	264	142	98	74	84	10	13	5	6	21	26	39	47	4	26	2147	
	10	8	5	77	46	94	145	105	98	55	81	23	25	9	9	21	42	37	13	2	24	1996	
	11	17	8	43	23	58	59	57	44	20	25	16	17	15	10	9	11	8	11	1	24	1015	32.6
	12	6	4	18	6	18	6	5	6	4	7	4	5	4	1	4	5	6	2	3	6	301	
	13	3	3	5			6		11		4	1		2		3	2		4	1	4	131	
	14		1	3	2		5		11		3				1	1		2	2	2		116	3.0
	15	2		7	2			10			3			1				2		1		53	
	16	2																				11	
	17			2				9														14	0.3
	18				2									1								6	
	19													1								7	
	20																						0.1
	21																					4	
	Total	61	35	250	193	442	662	475	546	393	350	75	70	49	37	87	177	194	213	27	188	10148	
	Mean	10.2	10.0	9.8	9.1	9.0	9.2	9.4	8.8	8.4	9.0	9.6	9.9	10.4	9.5	9.2	8.7	8.8	8.0	9.2	8.6	9.0	
0.333-mm mesh	4			2																1		5	
	5				3		6					2			1		1	2	16	7	3	68	3.1
	6	3	1		3	8	11	5		2	4	1	2	3		7	7	2	21	15	6	220	
	7	12	12	35	32	20	39	60	9	13	100	10	10	32	8	10	8	18	32	14	17	1366	
	8	6	7	35	33	67	169	129	11	65	87	10	18	27	2	21	30	63	49	20	22	2224	59.1
	9	9	2	29	33	103	164	120	18	28	105	12	14	21	11	17	33	46	46	14	13	1964	
	10	20	7	46	32	115	135	74	42	35	75	15	25	13	7	3	23	33	26	8	14	1886	
	11	8	7	51	20	43	40	46	13	17	26	10	3	3	8	7	7	7	18	7	13	835	32.8
	12		2	14	2	20		18	9	5	13	3	5		4	2	1	7	2	4	8	373	
	13			9	2	12			3	2	4	1	3	1	1			6	2	1		167	
	14	1									4	1		1		2			2	3	2	115	4.0
	15	1		2	2			5			13						2	2	2	1	1	95	
	16	1		7	2						4	2										58	
	17			2		8											1			1		18	0.9
	18							4														4	
	19																						
	20											1						1				5	0.1
	21																					1	
	Total	61	38	232	164	396	564	461	105	167	435	68	80	101	42	79	113	187	216	96	99	9404	
	Mean	9.3	8.8	9.8	9.0	9.6	8.8	9.1	9.7	9.0	9.0	9.4	9.2	8.3	9.3	8.8	8.9	9.0	8.3	8.4	8.9	9.1	

length groups are plotted in Fig. 3 and 4. The mean lengths for total 0.253- and 0.333-mm mesh samples were identical for 1.5-knot tows. A Kolmogorov–Smirnov Test (Hollander and Wolfe 1973) showed no significant differences in length frequency distributions for the two mesh sizes. For 3.5-knot tows the mean length of larvae was 0.1 mm lower for 0.253-mm mesh samples. The practical significance of such a small difference in mean length is difficult to evaluate and interpretation is complicated by asymmetry of the length distributions. However, examination of the shapes of the plots of numbers/1000 m^3 for 0.253- and 0.333-mm mesh nets (Fig. 3) indicates that while both meshes sample the same range of lengths, the lengths of greatest frequency are smaller for the finer mesh net. This is somewhat obscured by the steepness of the logarithmic scale of the abscissa. However, a Kolmogorov–Smirnov Test on length frequency showed the distribution of lengths to be significantly different ($\alpha = 0.005$) for the two mesh sizes.

ABUNDANCE OF LARVAL HERRING

Individual tow and average abundance values for 3-mm-length group and total herring larvae for 0.253- and 0.333-mm mesh tows at 3.5- and 1.5-knot stations are listed in Tables 5 and 6. The average abundances for 1-mm-length groups are plotted in Fig. 3 and 4. A *t*-test (Snedecor and Cochran 1967) of logarithmically transformed abundance data for 3-mm-size groups showed no mesh size related differences in the numbers of larvae retained at either towing speed (Tables 5 and 6).

The abundance of larval herring, as evidenced by both the 0.253- and 0.333-mm mesh catches, was appreciably greater during the initial 48-h sampling

TABLE 4. Length frequencies and mean lengths of larval herring for 0.253- and 0.333-mm mesh nets, 1.5-knot station.

	Length (mm)	Tow number 1	2	3	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
0.253-mm mesh	4												1												
	5				1					3	1	1	3	1				1		1	1	1			1
	6	2	2		1	1	1	1	2	8	9	6	2	2	1			9		3		3	2	2	5
	7	5	1		5	7	2	4	4	6	12	11	4	1	1		2	22	3	13	4	5	4	3	5
	8	14	10	4	6	2	6	6	10	18	31	31	13	18	13	5	6	11	12	29	12	17	12	3	7
	9	11	4	1	6	4	3	1		23	19	27	11	21	12	4	6	6	2	19	17	16	14		5
	10	6	8	3	7	12	5	9	14	10	21	20	10	15	10	9	3	2	6	11	7	9	15	2	6
	11	8	7	3	5	9	6	2	6	11	17	15	3	5	4	4	3		8	10	5	4	13	3	4
	12		3	3	3	2	5	7	10	5	7	2	1	9	6	10	2	1	2	6	2	3	2	1	5
	13	1	1		1		2				1	4	1	2	2	1			3	1	2		1		1
	14	1	1			1	1	1	2		1	1				1				1					
	15							2	1		1	1	2	1		1									
	16		1		1	1		2			3					1									
	17						2		1			2									1				
	18							1																	
	19											1													
	20																								
	21																								
	Total	48	38	14	36	39	33	36	50	84	123	122	51	75	49	36	22	52	36	94	51	58	63	14	39
	Mean	9.0	9.7	10.0	9.4	9.7	10.2	10.6	10.1	8.8	9.2	9.3	8.8	9.5	9.5	10.8	9.2	7.4	9.3	8.9	9.3	8.8	9.4	8.7	8.9
0.333-mm mesh	4										1		1												
	5							1			7	7										1		1	1
	6	2			1				1	6	7	8	2	1				2		1		4	4	2	3
	7	9	2	3	3	7	4	3	1	8	7	26			2	3	1	7	2	9	2	9	3	2	6
	8	9	10	2	7	8	7	7	7	22	40	22	11	14	12	1	5	8	9	24	13	18	7	1	9
	9	11	10	2	2	9	2	12	6	15	22	19	10	17	10	2	10	9	11	19	8	8	12	5	5
	10	3	5	5	10	12	9	7	12	11	25	22	6	16	7	5	9	13	8	21	8	7	15	3	12
	11	7	1	2	8	9	7	7	7	10	23	10	2	16	6	7	5	7	8	13	2	4	12	2	6
	12	4	1		4	5	4	2	5	5	15	5	8	9	7	7		2	2	12	3		5		2
	13			2		1			2	3	7	1		3		5	3	3	1	2			5		
	14						1	1		1	1		3	1		1			1	3					
	15					1			1				1							2					
	16			1	1		2	2	1																
	17				1		2									1									
	18						1																		
	19																								
	20																								
	21																								
	Total	45	29	17	37	52	39	42	43	81	155	120	44	77	44	32	33	51	42	106	36	51	63	16	44
	Mean	8.9	8.9	9.9	10.0	9.6	10.6	9.7	10.2	7.7	9.3	8.5	9.8	10.0	9.6	11.1	9.7	9.5	9.6	9.7	9.1	8.3	9.8	8.5	8.9

	Length (mm)	Tow number 26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	Total No.	Total %
0.253-mm mesh	4										2														3	
	5	1									2	4		3							1				26	4.9
	6	2	1	1	1					1	8	6	9	6			2								99	
	7	4	1	1		2	2	6	6	11	14	7	6	13	1			3	4	3	6	2	1		217	
	8	9	3	1	7	10	6	13	4	12	8	9	8	9	8	2	3	6	13	10	3	7	4	4	455	43.2
	9	17	3	4	3	9	3	11	6	13	18	10	15	10	8	8	4	11	9	19	7	12	7	9	448	
	10	10	3	8	7	14	13	9	9	13	28	20	15	17	14	9	8	15	10	20	10	7	4	11	494	
	11	5	3	9	6	13	7	9	5	8	53	22	11	7	7	7	5	10	8	19	3	4	4	8	388	44.5
	12	6	2	7	2	20	5	3	2	5	42	10	11	11	5	4	6	8	4	15		1	1	6	273	
	13	2	1	3	2	1	2		2	1	12	2	2	5	4	3	6	3	9	5	1	1		1	91	
	14	1				4				1		5					2	1	3	1			1	1	31	5.7
	15				2	1		1	1	1	4		2	1					2	1					25	
	16	1			3		1		1		2		2	1				1	1	1			1		24	
	17										4	2			1					1					14	1.6
	18										2				1										4	
	19																								1	
	20					1																			1	0.1
	21																									
	Total	58	17	34	33	75	39	52	36	66	199	97	81	83	49	33	36	58	62	95	31	34	23	40	2594	
	Mean	9.5	9.7	10.6	10.7	10.7	10.2	9.3	9.8	9.3	10.6	9.9	9.7	9.4	10.4	10.4	10.7	10.2	10.3	10.4	9.0	9.3	9.9	10.3	9.7	

TABLE 4 (*Concluded*)

0.333-mm mesh																									
4	1																							3	
5	2						1			2	1	4	2			1								32	4.6
6	4	1				1	2		1	11	4	7	8	1		1				1		2		88	
7	5	2	1	3	2		4	1	7	15	7	9	7	1	4	4		1	6	5	3	1		207	
8	13	5	4	11	4	2	12	6	8	21	10	16	11	9	2	7	14	7	22	15	9	2	3	486	42.4
9	13	4	5	9	7	4	12	6	22	31	13	12	6	8	3	5	6	6	13	10	11	2	8	452	
10	9	5	6	10	19	9	12	7	23	49	24	14	10	7	9	10	15	5	13	5	7	2	9	531	
11	9	5	9	3	23	11	11	2	16	35	28	11	10	3	7	5	9	10	16	5	4	3	5	421	45.9
12		2	4	3	17	6	4		8	38	20	10	4	7	4	4	8	15	12	5	3		8	289	
13		1	4	2	12	5	3	1		2	6	5		6	4		4	6	4	2	1	2		108	
14					3				2	6	2	1	2	2	1	1	4	2	1	1		1		42	6.0
15					1					2		2				1	1		1					13	
16	1						1		1			1				1	1			1				14	
17										2			1		1		1	1						10	1.1
18						1							2				1							5	
19																									
20																									
21																									
Total	57	25	33	41	88	39	62	23	88	214	115	92	63	44	35	40	65	53	88	51	38	15	33	2701	
Mean	8.8	9.5	10.4	9.4	11.0	10.7	9.6	9.3	9.8	10.5	10.8	9.4	9.2	10.2	10.5	9.7	10.7	10.9	9.8	9.3	9.3	9.7	10.2	9.7	

period (3.5-knot station). The length frequency and mean length data for larvae caught at the two towing speeds (Fig. 3 and 4) show that the difference in numbers was not due to an increase in the catch of larger larvae in the 3.5-knot tows (decrease in avoidance). The Great South Channel area is characterized by strong tidal currents and the average net residual drift is to the south. On a basis of drift bottle returns, Bumpus and Lauzier (1965) estimated that during October the

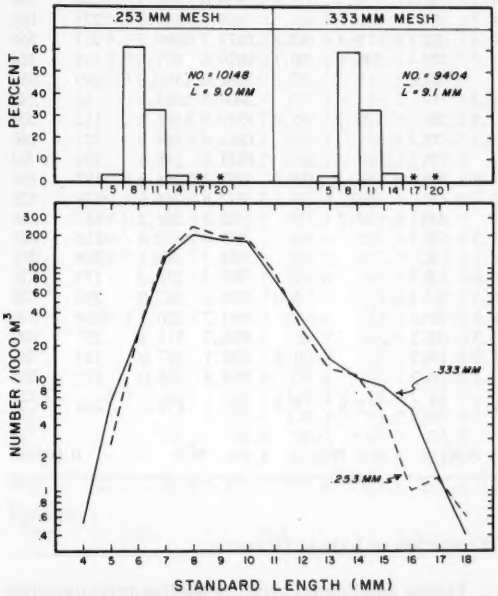

FIG. 3. Total catch, mean length, length frequency (3-mm size groups) and average abundance (1-mm size groups) of larval herring for 0.253- and 0.333-mm mesh nets, 3.5-knot tows * = less than one).

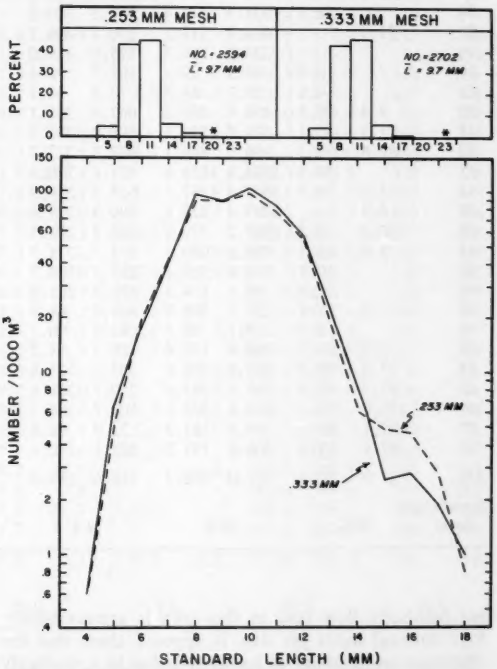

FIG. 4. Total catch, mean length, length frequency (3-mm size groups) and average abundance (1-mm size groups) of larval herring for 0.253- and 0.333-mm mesh nets, 1.5-knot tows (* = less than one).

TABLE 5. Abundance (no./1000 m³) of larval herring and zooplankton displacement volumes (cm³/1000 m³) for 0.253-mm and 0.333-mm mesh nets, 3.5-knot station.

Tow No.	4–6 mm		7–9 mm		10–12 mm		13–15 mm		16–18 mm		Total		Displacement volume	
	0.253	0.333	0.253	0.333	0.253	0.333	0.253	0.333	0.253	0.333	0.253	0.333	0.253	0.333
1	13.3	33.6	936.9	1083.9	431.9	500.0	13.3	124.2		13.4	1395.3	1765.1	249	201
2	103.4	15.2	808.4	688.2	360.2	566.2	195.4	342.2		98.9	1498.1	1711.0	364	228
3	65.1	13.7	914.4	836.2	263.7	320.8	78.8	47.8		13.7	1321.9	1232.1	274	205
5	13.1	25.1	386.9	442.0	334.4	291.5	124.6	59.6	19.7		878.7	812.8	262	188
6	38.2	16.3	500.0	469.0	149.7	270.4	38.2	68.4			726.1	824.1	334	163
7	29.0	156.4	809.1	987.7	539.4	497.9	70.5	53.5			1448.1	1699.6	705	247
8	50.0		800.0	663.6	327.3	470.1	59.1	92.2	13.6	27.7	1250.0	1253.5	273	184
10	20.7	30.0	1082.9	915.0	725.4	475.0	41.5	25.0			1870.5	1445.0	233	225
11	21.9	66.1	736.8	687.2	364.0	198.2		8.8	4.4		1127.2	960.4	197	176
13		32.4	736.5	536.0	664.3	482.0	28.9	21.6			1429.6	1071.9	144	54
14	4.2	8.5	245.8	597.5	165.3	250.0	4.2	25.4			419.5	881.4	127	127
15	17.1	16.9	529.9	286.9	307.7	211.0					854.7	514.8	171	139
16	4.9		365.9	299.0	302.4	274.5		4.9			673.2	578.4	220	270
17		10.6	439.2	494.7	476.2	398.9		21.3		10.6	915.3	936.2	212	213
18			818.8	880.8	416.1	470.2	13.4	26.5			1240.3	1377.5	101	199
19	33.8		1014.5	1117.1	536.2	692.7	29.0	63.4			1613.5	1878.1	290	293
20	19.6	34.7	303.9	341.6	29.4	9.9		5.0		5.0	352.9	396.0	147	248
21	12.9	13.0	193.1	238.1	8.6	4.3					214.6	255.4	322	325
22	12.4	50.6	539.4	632.9	49.8	42.2		8.4	8.3		618.3	734.2	332	211
23	62.1	40.4	758.7	670.0	41.4	84.2					862.1	794.6	259	135
24		28.9	721.5	574.4	367.1	305.8	12.7	49.6	12.7		1113.9	958.7	190	165
25	15.0		454.9	213.2	270.7	496.3	7.5	40.4			748.1	750.0	169	37
26			234.9	184.7	395.0	338.0	7.1	7.0		17.4	637.0	547.0	178	122
27		9.9	76.9	89.1	103.7	92.4	16.7	6.6	6.7	3.3	204.0	201.3	284	149
28		4.1	58.3	86.8	70.8	66.1	16.7				145.8	157.0	271	186
30	9.4	9.5	436.6	469.2	647.9	526.1	70.4	52.1	9.4	42.7	1173.7	1099.5	211	166
31	21.3	32.1	574.5	524.1	398.9	288.8	21.3	21.4	10.6	10.7	1026.6	877.0	319	214
32	87.6	32.3	996.0	766.1	677.3	717.7		48.2		32.3	1261.0	1596.8	219	161
33		74.9	1934.8	1638.8	921.1	770.9	48.2				2903.5	2484.6	66	242
34	15.0	18.7	1067.4	1157.3	625.5	516.9	37.5	18.7	33.7	15.0	1779.0	1726.6	112	206
35	16.9		1253.4	128.8	500.0	217.0	74.3	10.2			1844.6	359.9	321	186
36	130.4	7.2	1007.2	379.9	286.2	204.3		7.2			1423.9	598.6	109	90
37	9.9	13.3	736.8	970.1	371.7	378.7	32.9	69.8		13.3	1151.3	1445.2	197	199
38		10.8	114.8	115.1	159.3	100.7	3.7	7.2		7.2	277.8	244.6	278	126
39		7.4	86.1	154.4	176.0	121.8		11.1	3.7		262.2	295.2	187	166
40	3.8	11.6	57.3	308.9	106.9	61.8	11.5	7.7			187.0	390.0	210	162
41		4.7	75.1	99.5	93.9	90.1	4.7	4.7			173.7	199.1	258	251
42	3.5	24.7	168.4	169.6	119.3	77.7	14.0	7.1			305.3	279.2	175	35
44	47.4	40.2	568.4	356.8	305.3	155.8	10.5	10.1		5.0	931.6	567.8	290	176
45	37.2	17.5	537.1	557.0	210.7	206.1	16.5	35.1			801.7	820.2	248	219
46	151.9	156.1	611.8	535.9	109.7	194.1	25.3	25.3			898.7	911.4	295	190
47	19.1	88.1	45.8	183.9	22.9	72.8	15.3	19.2		3.8	103.1	367.8	324	96
48	87.5	33.5	406.8	193.3	205.3	130.1	15.2	11.2			714.8	368.0	475	260
Avg.	27.9	27.3	581.0	518.7	310.6	289.0	28.1	35.5	2.9	7.5	951.6	878.3	248	178
Significance level	N.S.		N.S.		N.S.		N.S.		N.S.		N.S.		0.005	

net southerly flow rate in this area is approximately four nautical miles per day. It appears, then, that the difference in numbers of larvae was due to a southerly shift in the larval herring population center during the 4-d sampling period.

CONDITION OF LARVAL HERRING

Tranter and Smith (1968) noted that pressure across the meshes (filtration pressure) influences the ultimate condition of the catch as well as the extent of extru-

TABLE 6. Abundance (no./1000 m³) of larval herring and zooplankton displacement volumes (cm³/1000 m³) for 0.253-mm and 0.333-mm mesh nets, 1.5-knot station.

Tow No.	4–6 mm		7–9 mm		10–12 mm		13–15 mm		16–18 mm		Total		Displacement volume	
	0.253	0.333	0.253	0.333	0.253	0.333	0.253	0.333	0.253	0.333	0.253	0.333	0.253	0.333
1	15.4	15.6	230.7	226.6	107.7	109.4	15.4				369.2	351.5	500	195
2	13.6		102.0	150.7	122.5	48.0	13.6		6.8		258.5	198.6	204	206
3			40.3	57.4	72.6	57.4		16.4		8.2	112.9	139.3	282	287
5	11.8	6.0	100.0	71.9	88.2	131.7	5.9		5.9	12.0	211.8	221.6	235	179
6	7.8		100.8	184.6	178.3	200.0	7.8	15.4	7.8		302.3	400.0	271	462
7	11.2		123.6	146.1	179.8	224.7	33.7	11.2	22.5	56.2	370.8	438.2	449	393
8	14.5	14.3	159.4	314.3	260.9	228.6	43.5	14.3	43.5	28.6	521.7	600.0	434	286
9	17.9	12.1	125.0	168.7	267.9	289.2	26.8	36.1	8.9	12.1	446.4	518.1	313	422
10	98.2	53.1	419.6	389.4	232.1	230.1		35.4			750.0	716.8	268	177
11	79.4	118.1	492.1	543.3	357.1	496.1	23.8	63.0	23.8		976.2	1220.5	318	118
12	73.7	157.9	726.3	705.2	389.5	389.5	63.2	10.5	21.1		1284.2	1263.1	211	105
13	69.0	34.5	321.8	241.4	160.9	183.9	34.5	46.0			586.2	505.8	115	230
14	28.9	9.7	384.6	301.0	278.8	398.1	28.9	38.8			721.2	747.6	144	97
15	11.9		309.5	266.7	238.1	222.2	23.8				583.3	488.9	60	111
16			105.8	70.6	270.6	223.5	35.3	70.6	11.8	11.8	423.5	388.2	235	235
17			123.9	141.6	70.8	123.9		26.6			194.7	292.0	221	133
18	73.0	14.6	284.7	175.2	21.9	160.6		21.9			379.6	372.3	110	183
19			163.5	213.6	153.9	174.8	28.9	19.4			346.2	407.8	289	243
20	39.2	9.8	598.0	509.8	264.7	451.0	19.6	68.6			921.6	1039.2	147	147
21	14.9		492.5	333.3	209.0	188.4	29.9		14.9		761.2	521.7	149	73
22	44.0	55.0	417.6	384.6	175.8	120.9					637.4	560.4	110	274
23	21.7	43.0	326.1	236.6	326.1	344.1	10.9	53.8			684.8	677.4	217	215
24	42.6	61.2	127.7	163.3	127.7	102.0					297.9	326.5	319	204
25	70.6	47.1	200.0	235.3	176.5	235.3	11.8				458.8	517.7	353	353
26	29.7	69.3	297.0	306.9	207.9	178.2	29.7		9.9	9.9	574.3	564.4	347	248
27	9.2	9.2	64.2	100.9	73.4	110.1	9.2	9.2			156.0	229.4	92	138
28	8.3		50.0	82.0	200.0	155.7	25.0	32.8			283.3	270.5	250	246
29	10.1		101.0	230.0	151.5	160.0	40.4	20.8	30.3		333.3	410.0	303	100
30			194.4	117.1	435.2	531.5	55.6	144.1			694.4	792.8	324	270
31		8.6	95.7	51.7	217.4	224.1	17.4	43.1	8.7	8.6	339.1	336.2	435	285
32		30.6	31.3	285.7	218.8	275.5	10.4	30.6		10.2	541.7	632.7	313	306
33			307.7	228.1	307.7	157.9	57.7	17.5	19.2		692.3	403.5	577	263
34	10.1	9.7	363.6	359.2	262.6	456.3	30.3	19.4		9.7	666.6	854.4	101	243
35	97.6	104.0	325.2	536.0	1000.0	976.0	130.1	80.0	65.0	16.0	1617.9	1712.0	528	320
36	98.0	48.5	254.9	291.3	509.8	699.0	68.6	77.7	19.6		951.0	1116.5	196	243
37	94.7	115.8	305.3	389.5	389.5	368.4	42.1	84.2	21.1	10.5	852.6	968.4	263	105
38	108.4	117.7	385.5	282.4	421.7	282.4	72.3	23.5	12.1	35.3	1000.0	741.2	241	177
39		11.1	197.7	200.0	302.3	188.9	46.5	88.9	23.3		569.8	488.9	233	278
40			109.9	94.7	219.8	210.5	33.0	52.6		10.5	362.6	368.4	55	211
41	19.8	19.0	69.3	152.4	188.1	181.0	79.2	19.0		9.5	356.4	381.0	198	191
42			124.2	121.2	205.0	200.0	24.9	54.6	6.2	18.2	360.3	393.9	217	182
43			151.2	80.0	127.9	171.4	75.6	45.7	5.8	5.7	360.5	302.9	204	200
44			178.8	222.8	301.7	222.8	39.1	32.6	11.2		530.7	478.3	307	217
45	6.7	6.6	106.7	197.4	86.7	98.7	6.7	19.7		6.6	206.7	335.5	100	197
46			166.7	178.3	95.2	108.5	7.9	7.8			269.8	294.6	119	194
47		14.5	87.6	36.2	65.7	36.2	7.3	21.7	7.3		167.9	108.7	73	36
48			270.8	215.7	520.8	431.4	41.7				833.3	647.1	208	196
Avg.	25.1	24.3	220.7	225.6	227.5	244.4	29.0	32.1	8.2	5.7	511.0	532.2	243	215
Significance level	N.S.		N.S.		N.S.		N.S.		N.S.		N.S.		N.S.	

sion. Because filtration pressure varies with the square of the approach velocity, small changes in towing speed will lead to relatively large changes in filtration pressure. In accordance with these observations, the condition of all size herring larvae was notably better in the 1.5-knot tow collections. This was especially true

in the case of early stage larvae. Only 3% of the yolk-sac larvae caught in 3.5-knot tows had their yolk sacs intact, while 50% of the yolk-sac larvae caught in 1.5-knot tows were undamaged. The extent of damage due to filtration pressure is important to several aspects of sample processing, including identification and measurement.

Zooplankton Biomass

Individual tow and average zooplankton displacement volumes for 0.253- and 0.333-mm mesh tows at 3.5- and 1.5-knot stations are listed in Tables 5 and 6. At a towing speed of 3.5 knots the 0.253-mm mesh displacement volumes were significantly greater than the 0.333-mm mesh displacement volumes. At a towing speed of 1.5 knots the difference in displacement volumes between the two mesh sizes was not significant. Extrusion of zooplankton through the 0.333-mm meshes was appreciably greater at 3.5 knots.

Zooplankton Abundance and Size Composition

Minimum, maximum, and mean lengths and abundances of invertebrate taxa for both mesh sizes from 10 randomly selected 3.5- and 1.5-knot tows are given in Table 7. For both 3.5- and 1.5-knot tows the 0.253-mm mesh catches of adult *Oithona* spp. and *Pseudo-Paracalanus* spp., unidentified copepodites, adult and juvenile *Hyperia* spp., and total invertebrates were significantly greater at the 2% level. The 3.5-knot, 0.253-mm mesh catches of adult and furcilia *Meganyctiphanes norvegica* and adult and juvenile *Tomopteris* spp. and the 1.5-knot, 0.253-mm mesh catches of adult *Alteutha depressa* were significantly greater at the 5% level. Sample sizes, however, were very small for the latter three taxa.

The ratios of mean abundances (0.253/0.333 mm mesh) were greatest for the smaller and more abundant taxa. This accounts for the appreciably smaller total taxon mean length values for 0.253-mm mesh samples and that the number of organisms increased with decreasing mesh size at a much greater rate than indicated by the displacement volume ratios (Tables 5 and 6). The highest 0.253/0.333 mm mesh abundance ratio was for unidentified copepodites in 1.5-knot tows.

Statistics for individual taxa were calculated for both mesh sizes and towing speeds using the dominance–frequency method of Fager and McGowan (1963). These statistics are summarized in Table 8. The taxa are listed sequentially on a basis of their average ranks.

TABLE 7. Length and abundance (no./m³) of zooplankton taxa for randomly selected

					Tow number (3.5-knot station)									
		Length (mm)			1		5		10		11		16	
Taxon	Stage[a]	Min.	Max.	Mean	0.253	0.33	0.2353	0.333	0.253	0.333	0.253	0.333	0.253	0.333
Copepoda														
Alteutha depressa	A	0.7	1.1	1.0	6.8		3.4	0.4		1.9		2.3		
Calanus finmarchicus	A	1.8	2.6	2.2		2.2		1.2		1.9		4.5	5.0	3.8
Calanus spp.	C	0.7	2.4	1.5	13.6	3.4	3.4	4.0	45.1	0.6		6.8	30.0	18.8
Candacia armata	A	1.7	2.4	2.0		28.4	10.1	15.3	2.7	11.5	98.8	1.1		2.5
	C	0.4	2.0	1.0	156.5	47.3	77.2	41.7	66.3	63.4	103.3	68.8	30.0	12.6
Centropages typicus	A	0.9	1.5	1.2		58.0	60.4	43.3	90.2	35.2	44.9	11.3	20.0	13.8
Centropages spp.	C	0.5	1.1	0.8	326.6	6.4	97.4	44.9	87.5	44.8		134.2	25.0	8.8
Metrida lucens	A	1.8	2.2	1.9	6.8	15.5	10.1	4.4				1.1	15.0	56.5
	C	0.5	1.7	1.1	68.0	10.3	36.9	18.1	23.9	41.0	22.5	42.9	244.8	104.2
Oithona similis and *spinirostris*	A	0.3	0.7	0.5	61.2	11.2	20.1	2.0	37.1	3.2		5.6	134.9	27.6
Pseudo-Paracalanus[b]	A	0.5	1.0	0.8	170.1	12.9	228.3	41.7	944.4	19.8	561.4	33.8	69.9	12.6
Unidentified	C	0.3	1.0	0.7	2830.5	11.6	1161.7		114.1	32.0	1491.1	28.2	989.0	27.6
Chaetognatha														
Sagitta spp.	A&J	2.5	15.6	8.2	34.0	7.3		10.4		2.6	13.5	12.4	30.0	21.4
Cladocera														
Evadne, Podon, Penilia spp.	A	0.4	1.2	0.9	6.8	4.3		3.6	2.7	7.0	22.5	14.7	10.0	
Amphipoda														
Hyperia spp.	A&J	0.6	3.2	1.4	74.8	12.0		3.2		5.8	13.5	9.0	10.0	5.0
Euphausiacea														
Meganyctiphanes norvegica	A&F	1.5	4.7	3.3	13.6		6.7	0.4		1.3		2.3		
Annelida														
Tomopteris spp.	A&J	1.2	13.1	4.7	6.8	3.4		3.6			4.5	4.5	5.0	
Total[c]			0.253 0.333	0.9 1.8	3776.2	235.0	1715.6	239.2	1416.6	272.0	2393.8	383.4	1633.4	316.2

The relative abundances of the more numerous taxa differed between mesh sizes and tow speeds as in the case of absolute abundance values (Table 7). The order of rank of the first seven taxa (unidentified copepodites – adult *Oithona* spp.) for 0.253-mm mesh samples was identical at both towing speeds, indicating that a similar assemblage of invertebrates was present during both sampling periods, although their absolute abundance, as in the case of larval fishes, was appreciably lower during the 1.5-knot station period. For 0.333-mm mesh samples, the rankings of the first three taxa were dissimilar at the two towing speeds. This variation was brought about by an upward shift in the ranks of the two smaller taxa (adult *Pseudo-Paracalanus* and *Metridia lucens* copepodites) at the lower towing speed. The order of rank of the next five taxa (*Centropages* spp. copepodites – adult *M. lucens*) was identical at both towing speeds.

Unidentified copepodites, which were the highest ranked taxon in 0.253-mm mesh tows ranked sixth in the 0.333-mm mesh tows. At the 3.5-knot station unidentified copepodites were dominant in 8/10 of the 0.253-mm mesh samples, but in none of the 0.333-mm mesh samples. At the 1.5-knot station this taxon was dominant in 7/10 of the 0.253-mm mesh samples and in 1/10 of the 0.333-mm mesh samples. The only other taxon making up 50% or more of the individuals in a sample was adult *Pseudo-Paracalanus* which was dominant in 2/10 of the 0.253-mm mesh, 3.5-knot samples and in 1/10 of the 0.253-mm mesh, 1.5-knot samples.

The observed variations in invertebrate retention rates resulted in large part from differences in mesh aperture size, although catching efficiency was also influenced by tow speed. With the exception of Amphipoda, all taxa collected in significantly greater numbers (2% level) by the 0.253-mm mesh net had a mean length of less than 1 mm (Table 7). The retention of copepods is mainly dependent on the relative size of the mesh and body width of the organism (widest part of the cephalothorax), but is contingent to some extent on differences in morphology, compressibility, and behavior (Vannucci 1968). In Table 9 we have summarized cephalothorax length and width measurements of the smaller copepod taxa and developmental stages. These measurements were obtained from other collections and from other areas within the Gulf of Maine, but the range and mean length values for the various taxa are similar to those observed on the subject cruise.

paired 0.253-mm and 0.333-mm mesh samples, 3.5-knot and 1.5-knot stations.

Tow number (3.5-knot station)														
20		30		40		44		48		Mean		Abundance ratio		
0.253	0.333	0.253	0.333	0.253	0.333	0.253	0.333	0.253	0.333	0.253	0.333	0.253/0.333	DF[d]	t^e
			0.6				6.4	3.9		1.4	1.2	1.2	6	1.6475
			6.1	1.0	4.9	5.4	5.2			1.2	3.0	0.4	9	0.1924
	1.3	4.8	12.7	4.9	13.8		14.2			10.2	7.6	1.3	13	1.7514
	3.1	33.7	7.3	2.0	3.0	5.4	7.7	11.7	20.0	16.4	10.0	1.6	15	0.7580
32.6	22.8	43.3	57.0	3.9	16.8	37.7	84.9	27.3	20.5	57.8	43.6	1.3	18	0.3215
105.4	138.8	38.5	26.7	1.0	12.9	53.9	74.6	66.2	47.1	48.1	46.2	1.0	17	0.0109
70.3	18.4	19.2	31.6	5.8	4.9	59.3	91.3	128.5	53.8	82.0	43.9	1.9	17	1.3108
		14.4	51.6	27.4	40.5		3.9		0.5	5.9	17.4	0.3	11	0.5946
27.6	9.5	33.6	49.1	55.7	168.0	70.1	43.7	3.9	8.6	58.7	49.5	1.2	18	0.3296
17.6		19.2	21.2	26.4	18.8	43.1	16.7	23.4		38.3	10.6	3.6	15	3.1501**
958.8	154.0	365.4	27.9	70.4	26.7	393.4	88.8	186.9	155.1	394.9	57.3	6.9	18	4.7266**
341.3	41.8	1139.4	20.6	309.7	78.1	1697.7	48.9	1857.2	27.6	1193.2	31.6	37.8	17	8.8731**
	1.9	24.0	18.8	1.0	34.6	5.4	19.1	7.8	2.4	12.9	13.0	1.0	15	0.3054
37.6	23.4	9.6	5.5	1.0	6.0		21.9	35.1	3.9	12.5	9.0	1.4	15	0.3441
	1.3	9.6	6.7		11.9	5.4	12.9	31.2	2.4	14.5	7.1	2.0	14	2.4066**
			4.9	2.0	1.0		2.6			2.2	1.3	1.7	7	2.0107*
							2.6			1.6	1.4	1.1	5	2.5784*
1591.2	419.51	754.7	350.0	512.0	441.8	2376.8	544.2	2386.7	347.8	1955.7	354.9	5.5	18	8.7998**

TABLE 7

Taxon	Stage[a]	Length (mm) Min.	Max.	Mean	Tow number (1.5-knot station) 13 0.253	13 0.333	18 0.253	18 0.333	23 0.253	23 0.333	25 0.253	25 0.333	27 0.253	27 0.333
Copepoda														
Alteutha depressa	A	0.4	1.1	0.9	1.5	1.5			2.8	1.0				0.3
Calanus finmarchicus	A	1.8	2.9	2.2	2.2	1.5	1.9	0.7		0.7		0.8	0.9	1.5
Calanus spp.	C	0.7	2.0	1.6	8.1	11.0		4.2	13.9	0.7			0.6	5.9
Candacia armata	A	1.7	2.6	2.1	2.9	8.8	9.3	3.5	11.1	2.8	3.0	1.5	1.8	2.0
	C	0.3	2.0	1.0	10.3	19.9	52.3	14.5	16.7	15.5	12.1	9.4	3.2	7.6
Centropages typicus	A	1.0	1.5	1.3	18.4	37.5	29.9	7.7	27.8	37.2	27.1	25.2	0.6	2.6
Centropages spp.	C	0.4	1.2	0.9	22.1	33.1	39.2	7.9	52.9	32.7	33.1	18.1	0.3	2.9
Metridia lucens	A	1.8	2.3	2.0	1.5	0.7		7.0				1.1	3.8	6.2
	C	0.5	1.8	1.2	16.9	16.2		18.9	55.7	10.3		3.8	9.1	20.3
Oithona similis and *spinirostris*	A	0.3	0.8	0.6	16.2	4.4	13.1	3.0	8.4	0.7	3.0	0.4	1.5	2.4
Pseudo-Paracalanus[b]	A	0.5	1.0	0.9	117.0	132.4	151.4		108.5	33.7	774.0	117.5	34.6	19.7
Unidentified	C	0.4	0.9	0.7	328.8	12.5	1126.8		1065.7	19.6	605.4	9.0	110.4	4.1
Chaetognatha														
Sagitta spp.	A&J	2.5	17.2	9.2	4.4	10.3	9.3	4.7	11.2	2.8	3.0	1.9	0.9	5.9
Cladocera														
Evadne, Podon, Penilia spp.	A	0.5	1.2	0.9	2.9	1.5	20.5	3.7		1.7	3.0	2.3	0.6	2.9
Amphipoda														
Hyperia spp.	A&J	0.6	6.7	2.1	2.2	2.9	7.5	2.3	5.6	2.8	9.0	3.0	1.2	
Euphausiacea														
Meganyctiphanes norvegica	A&F	1.9	5.7	4.0	0.7	0.7		1.2	5.6	2.1				0.6
Annelida														
Tomopteris spp.	A&J	1.2	15.2	6.3		8.8		0.7		0.7	3.0		0.9	1.2
Total[c]			0.253	0.9										
			0.333	1.6	556.1	303.8	1468.7	80.1	1385.7	164.8	1475.8	198.0	170.3	86.3

[a]A = adult, C = copepodite, F = furcilia, J = juvenile.
[b]*Pseudocalanus minutus* and *Paracalanus parvus*.
[c]Includes infrequently occurring taxa not specified in table.
[d]Degrees of freedom.
[e]L_n transformed, * $\alpha = 0.05$, ** $\alpha = 0.02$.

The mesh diagonals of the 0.333- and 0.253-mm mesh nets are 0.47 and 0.36 mm, respectively (Fig. 2). A comparison of these values with maximum cephalothorax width values shows that with the exception of adult *Oithona* spp., the observed taxon differences in retention rates are plausible in terms of the "diagonal rule." As would be expected, negligible numbers of copepod nauplii were retained by either mesh net. However, adult *Oithona* spp. were retained by both mesh nets in far greater numbers than would be predicted on the basis of their cephalothorax width. Saville (1958) observed that the 50% retention point of *Oithona* is a much smaller size than for other copepods of approximately the same dimensions and concluded that the most likely explanation for this anomaly was that in *Oithona* 90% of the specimens had their antennae extended away from the body in contrast to other copepod species, in which the antennae were closely pressed along the body.

The body dimensions of Amphipoda (*Hyperia* spp.), which was the only other taxon collected in significantly (2% level) greater numbers by the 0.253-mm mesh net, are appreciably greater than those of the copepod taxa having high 0.253-mm/0.333-mm mesh abundance ratios. Although *Hyperia* spp. have rounder bodies than most, they, like all amphipods, are laterally compressed, so that body depth rather than width is the critical dimension regarding mesh retention. Length, width, and depth measurements (in millimetres) of a random sample of adult and juvenile *Hyperia* spp. from 0.333- and 0.253-mm mesh samples are:

No.	Length Range	Length Mean	Width Range	Width Mean	Depth Range	Depth Mean
59	0.9–6.8	1.84	0.3–1.5	0.47	0.3–1.6	0.52

The mean width of *Hyperia* spp. was equal to, and the mean depth was slightly greater than, the 0.333-mm mesh diagonal (0.47 mm). Both body dimensions were

(*Concluded*)

Tow number (1.5-knot station)														
28		31		38		41		43		Mean		Abundance ratio		
0.253	0.333	0.253	0.333	0.253	0.333	0.253	0.333	0.253	0.333	0.253	0.333	0.253/0.333	DF[d]	t[e]
1.1				10.8	2.6	10.1	0.6			2.6	0.6	4.3	8	2.1827*
5.3	7.9	2.2	6.4			10.1	1.8	3.7	16.5	2.6	3.8	0.7	14	0.5045
28.8	22.0	4.5	2.8		1.5	15.2	9.1	11.9	2.2	8.3	5.9	1.4	14	1.0623
2.1	4.2	15.6	9.1		0.8	10.1	6.1	0.7	4.8	5.6	4.4	1.3	17	0.4541
20.3	22.6	46.8	11.9	9.3	2.3	55.8	23.2	20.8	21.6	24.8	14.9	1.7	18	0.9912
6.4	12.6	8.9	5.5	18.5	18.8	23.4	12.2	5.2	10.6	16.6	17.0	1.0	18	0.2817
7.5	7.9	33.4	7.7	20.1	20.3	81.1	9.1	8.9	9.5	29.9	14.9	2.0	18	0.6351
18.1	17.3	40.1	27.9			10.1	6.7	6.0	24.3	8.0	9.0	0.9	12	0.3243
64.0	84.5	91.3	33.9		0.8	50.7	37.8	75.2	64.0	36.3	29.1	1.2	15	1.5480
13.9	3.2	37.9	5.2	3.1	0.8	10.1	3.1	12.7	6.2	12.0	3.0	4.0	18	3.1783**
75.7	32.0	454.1	12.1	106.4	73.4	207.8	67.7	28.3	21.2	205.8	51.0	4.0	17	2.5523**
380.8	34.6	402.9	1.9	917.6	21.8	1302.8	17.1	190.5	2.6	643.2	12.3	52.3	17	9.3995**
8.6	13.2	8.9	4.1	9.3	1.1	20.3	6.7	7.5	9.9	8.4	6.1	1.3	18	0.8392
9.6	8.4	33.4	5.5	6.1	1.2	5.1	6.1	5.2	4.4	8.7	3.7	2.3	17	1.3542
6.4	4.2	13.4	3.0	4.6	1.1	5.1	1.2	4.5	1.5	5.9	2.3	2.6	17	2.9141**
1.1	2.1			1.5	0.4	10.1	1.8	2.2		2.1	0.9	2.3	11	1.6237
1.1	1.6		3.1		0.4			1.5	2.6	0.6	1.9	0.3	10	0.0686
650.7	278.0	1193.2	142.3	1107.7	147.6	1830.0	210.9	385.5	206.3	1022.4	181.8	5.6	18	5.8452**

appreciably greater than the 0.253-mm mesh diagonal (0.36 mm). The combination of a lack of long appendages, greater compressibility, and a behavior pattern similar to chaetognaths, euphausiids, and fishes (active escapement) most likely accounts for the proportionally greater loss of amphipods than of copepods of similar length (e.g. *M. lucens*) through the 0.333-mm meshes.

Food of Larval Herring

None of the 4–6-mm (yolk-sac) larvae examined had food items in their guts. The feeding incidence of the larger length groups and of total larval herring at the two towing speeds is summarized in Table 10. Feeding incidence was higher for larvae caught in 1.5-knot station tows. The tow speed related differences in the percentage of larvae with food items were greatest for 7–9- and 13–15-mm size groups. It has been suggested that possibly the larvae were not feeding at as high a rate during the 3.5-knot sampling period, but the most logical reason for the differences in feeding incidence would appear to be increased defecation or regurgitation (Hardy 1924) associated with increased filtration pressure and turbulence at the higher towing speed.

The numbers and percentages of zooplankton taxa in the guts of 3-mm length group and total larvae from combined 1.5- and 3.5-knot samples are given in Table 11. Food items eaten by 7–9-mm larvae consisted mainly of copepod copepodites and adults (10–12-mm larvae, 50 and 27%, respectively; 13–15-mm larvae, 59 and 26%, respectively). Invertebrate eggs, although not a major food item, were ingested by all size larvae. There was a decrease in the percentage occurrence of copepod nauplii and an increase in the percentage occurrence of copepod copepodites with increasing larval length. The proportion of adult copepods in the alimentary tracts of 7–9-mm larvae (12% of total food items) was appreciably less than in 10–12- and

13–15-mm larvae (27 and 26% of total food items, respectively). Size is a most significant factor in the selection of food organisms.

A comparison of food ingested to that available is not applicable here, for the majority of taxa eaten by larval herring were either not retained or not fully retained by either the 0.253- or 0.333-mm mesh net. A comparison of the percent composition of zooplankton taxa collected in the five 0.253- and 0.333-mm mesh, 1.5- and 3.5-knot daytime tows selected for gut analyses with the percent composition of zooplankton taxa in the alimentary tracts of larval herring from combined tows (Fig. 5) clearly illustrates this disparity in the data.

For 7–9-mm larvae, 63% of the food items were not retained in either mesh net. Unidentified copepodites, which accounted for 31% of the food items for this size group, were to a large extent extruded through the 0.333-mm mesh. For 10–12-mm larvae, the gut contents consisted of taxa, 48% of which were not retained by either mesh net. *Oithona* spp. adults, which accounted for 17% of the items eaten by this size group, made up less than 2% of the 0.253- and 0.333-mm mesh catches. For 13–15-mm larvae, 37% of the food items were not retained by either mesh net. *Oithona* spp. adults made up 19% of the total number of food items ingested by this size group. The 0.253- and 0.333-mm mesh samples are obviously not representative of the composition of zooplankton taxa which are the primary food of early stage larval herring.

Conclusions

To date, no generalizations can be made on the relationship of mesh size and towing speed to the retention rates of plankton. On the basis of our limited observations, however, it appears that the 1.5-knot tow was adequate to sample the larval herring populations encountered (<21 mm) in the area of study and that the effectiveness of invertebrate zooplankton sampling was increased at the lower towing speed because:

1) There was no evidence in the data of increased avoidance of the gear by larger herring larvae at the lower towing speed.

2) There were no significant mesh-size related differences in the length frequency distributions and abundances of larval herring in the 1.5-knot tow collections.

3) The condition of larvae, especially in the yolk-sac stage, was markedly better in the 1.5-knot tow collections.

4) Larval feeding incidence was appreciably higher at 1.5 knots.

TABLE 8. Relative abundance of zooplankton taxa for randomly selected paired 0.253- and 0.333-mm mesh samples, 3.5- and 1.5-knot stations.

3.5-knot station										
0.253-mm mesh						0.333-mm mesh				
Taxon	Stage[a]	Average rank[c]	Dominance[d]	Abundance[e]	Frequency[f]	Taxon	Stage	Average rank	Dominance	Abundance	Frequency
Unidentified	C	16.80	8/10	114.1–2830.5 (1472.3)	10/10	*Candacia armata*	C	14.20	0/10	12.6–84.9 (48.7)	10/10
Pseudo-Paracalanus[b]	A	15.90	2/10	69.9–958.8 (513.4)	10/10	*Pseudo-Paracalanus*	A	13.90	0/10	12.6–155.1 (83.8)	10/10
Candacia armata	C	12.70	0/10	3.9–156.5 (80.2)	10/10	*Metridia lucens*	C	13.50	0/10	8.6–168.0 (88.3)	10/10
Centropages spp.	C	12.35	0/10	5.8–326.6 (166.2)	9/10	*Centropages typicus*	A	13.40	0/10	11.3–138.8 (75.0)	10/10
Metrida lucens	C	12.10	0/10	3.9–244.8 (124.3)	10/10	*Centropages* spp.	C	12.95	0/10	4.9–134.2 (69.5)	10/10
Centropages typicus	A	11.35	0/10	1.0–105.4 (53.2)	9/10	Unidentified	C	12.05	0/10	11.6–78.1 (44.8)	9/10
Oithona spp.	A	10.65	0/10	17.6–134.9 (76.2)	9/10	*Sagitta* spp.	A&J	10.05	0/10	1.9–34.6 (18.2)	10/10
Candacia armata	A	8.00	0/10	2.0–98.8 (50.4)	7/10	*Metridia lucens*	A	8.75	0/10	0.5–56.5 (28.5)	8/10
Cladocera	A	7.65	0/10	1.0–37.6 (19.3)	8/10	Cladocera	A	8.40	0/10	3.6–23.4 (13.5)	9/10
Sagitta spp.	A&J	7.65	0/10	1.0–34.0 (17.5)	7/10	*Oithona* spp.	A	8.35	0/10	2.0–27.6 (14.8)	8/10
Calanus spp.	C	7.05	0/10	3.4–45.1 (24.2)	6/10	*Candacia armata*	A	8.35	0/10	1.1–28.4 (14.7)	10/10
Hyperia spp.	A&J	7.00	0/10	5.4–74.8 (40.1)	6/10	*Hyperia* spp.	A&J	8.00	0/10	1.3–12.9 (7.1)	10/10
Metridia lucens	A	6.45	0/10	6.8–27.4 (17.0)	5/10	*Calanus* spp.	C	7.35	0/10	0.6–18.8 (9.7)	9/10
Meganyctiphanes norvegica	A&F	4.90	0/10	2.0–13.6 (7.8)	3/10	*Calanus finmarchicus*	A	4.35	0/10	1.2–6.1 (3.6)	8/10
Calanus finmarchicus	A	4.20	0/10	1.0–5.4 (3.2)	3/10	*Tomopteris* spp.	A&J	3.25	0/10	2.6–4.5 (3.5)	4/10
Alteutha depressa	A	4.15	0/10	3.4–6.8 (5.1)	3/10	*Alteutha depressa*	A	3.10	0/10	0.4–6.4 (3.4)	5/10
Tomopteris spp.	A&J	4.10	0/10	4.5–6.8 (5.6)	3/10	*Meganyctiphanes norvegica*	A&F	2.85	0/10	0.4–4.9 (2.6)	6/10

TABLE 8 (*Concluded*)

1.5-knot station											
Unidentified	C	16.80	7/10	110.4–1302.8 (706.6)	10/10	*Pseudo-Paracalanus*	A	14.60	1/10	12.1–132.4 (72.2)	9/10
Pseudo-Paracalanus	A	16.10	1/10	28.3–774.0 (401.1)	10/10	*Metridia lucens*	C	14.40	0/10	0.8–84.5 (42.6)	10/10
Candacia armata	C	13.05	0/10	3.2–55.8 (29.5)	10/10	*Candacia armata*	C	14.20	0/10	2.3–23.2 (12.7)	10/10
Centropages spp.	C	12.05	0/10	0.3–81.1 (40.7)	10/10	*Centropages typicus*	A	12.95	0/10	2.6–37.5 (20.0)	10/10
Metridia lucens	C	11.25	0/10	9.1–91.3 (50.2)	7/10	*Centropages* spp.	C	12.55	0/10	2.9–33.1 (18.0)	10/10
Centropages typicus	A	10.55	0/10	0.6–29.9 (15.2)	10/10	Unidentified	C	10.65	0/10	1.9–34.6 (18.2)	9/10
Oithona spp.	A	10.15	0/10	1.5–37.9 (19.7)	10/10	*Sagitta* spp.	A&J	10.05	0/10	1.1–13.2 (7.1)	10/10
Sagitta spp.	A&J	9.35	0/10	0.9–20.3 (10.6)	10/10	*Metridia lucens*	A	9.15	0/10	0.7–27.9 (14.3)	8/10
Calanus spp.	C	7.75	0/10	0.6–28.8 (14.7)	7/10	*Calanus* spp.	C	8.40	0/10	0.7–22.0 (11.3)	9/10
Cladocera	A	7.65	0/10	0.6–33.4 (17.0)	9/10	Cladocera	A	8.35	0/10	1.2–8.4 (4.8)	10/10
Hyperia spp.	A&J	7.40	0/10	1.2–13.4 (7.3)	10/10	*Candacia armata*	A	8.15	0/10	0.8–9.1 (4.9)	10/10
Candacia armata	A	7.20	0/10	0.7–15.6 (8.1)	9/10	*Oithona* spp.	A	6.35	0/10	0.4–6.2 (3.3)	10/10
Metridia lucens	A	7.10	0/10	1.5–40.1 (20.8)	6/10	*Calanus finmarchicus*	A	6.05	0/10	0.7–16.5 (8.6)	9/10
Calanus finmarchicus	A	5.05	0/10	0.9–10.1 (5.5)	7/10	*Hyperia* spp.	A&J	6.00	0/10	1.1–4.2 (2.6)	9/10
Aiteutha depressa	A	4.15	0/10	1.1–10.8 (5.9)	5/10	*Tomopteris* spp.	A&J	4.10	0/10	0.4–8.8 (4.6)	8/10
Meganyctiphanes norvegica	A&F	3.85	0/10	0.7–10.1 (5.4)	6/10	*Aiteutha depressa*	A	3.55	0/10	0.3–2.6 (1.4)	5/10
Tomopteris spp.	A&J	3.55	0/10	0.9–3.0 (1.9)	4/10	*Meganyctiphanes norvegica*	A&F	3.50	0/10	0.4–2.1 (1.2)	7/10

[a]A = adult, C = copepodite, F = furcilia, J = juvenile.
[b]*Pseudocalanus minutus* and *Paracalanus parvus*.
[c]Taxa were ranked within each sample on a basis of numbers of individuals. Ranks for each taxon were averaged over the total samples.
[d]Proportion of samples in which taxon was among those making up 50% of the individuals.
[e]Range and median (in parentheses) of number of individuals per m^3 in sample in which taxon was found.
[f]Proportion of total samples in which taxon was found.

TABLE 9. Copepod cephalothorax length–width measurements.

Taxon	Stage[a]	No.	Length (mm)		Width (mm)	
			Range	Mean	Range	Mean
Oithona spp.	A	55	0.28–0.80	0.47	0.12–0.28	0.17
Pseudo-Paracalanus	A	55	0.64–1.08	0.83	0.20–0.40	0.29
Unidentified	C	114	0.28–1.08	0.63	0.08–0.36	0.21
Unidentified	N	76	0.08–0.80	0.39	0.04–0.34	0.17

[a]A = adult, C = copepodite, N = nauplii.

TABLE 10. Feeding incidence of larval herring by length groups and speed of tow.

	Length (mm)							
	7–9		10–12		13–15		Total	
	No. examined	% with food	No. examined	% with food	No. examined	% with food	No. examined	% with food
3.5-knot station	115	32	77	77	8	38	200	50
1.5-knot station	68	53	107	79	22	86	197	71

TABLE 11. Food of larval herring.

Food items	Length (mm) 7–9 No.	7–9 %	10–12 No.	10–12 %	13–15 No.	13–15 %	Total No.	Total %
Invertebrate eggs	10	6.9	31	9.4	5	7.1	46	8.4
Copepod nauplii	61	42.1	44	13.3	6	8.6	111	20.4
Copepodite stages								
Oithona spp.	6		39		7		52	
Pseudo-Paracalanus spp.	9		37		6		52	
Unidentified	42		90		28		160	
Total	57	39.3	166	50.3	41	58.6	264	48.4
Adult copepods								
Oithona spp.	5		52		12		69	
Pseudo-Paracalanus spp.	4		20				24	
Unidentified	8		17		6		31	
Total	17	11.7	89	27.0	18	25.7	124	22.8
Total number of food particles	145		330		70		545	

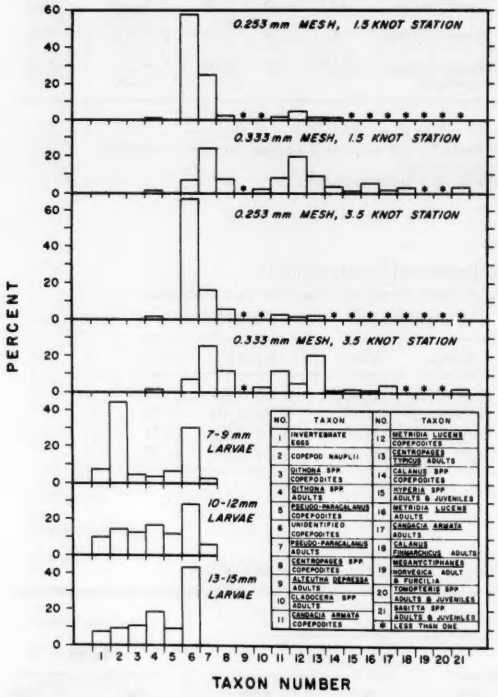
0.253 mm MESH, 1.5 KNOT STATION
0.333 mm MESH, 1.5 KNOT STATION
0.253 mm MESH, 3.5 KNOT STATION
0.333 mm MESH, 3.5 KNOT STATION
7-9 mm LARVAE
10-12mm LARVAE
13-15mm LARVAE
PERCENT
TAXON NUMBER
NO. TAXON
1 INVERTEBRATE EGGS
2 COPEPOD NAUPLII
3 OITHONA SPP COPEPODITES
4 OITHONA SPP ADULTS
5 PSEUDO-PARACALANUS COPEPODITES
6 UNIDENTIFIED COPEPODITES
7 PSEUDO-PARACALANUS ADULTS
8 CENTROPAGES SPP COPEPODITES
9 ALTEUTHA DEPRESSA ADULTS
10 CLADOCERA SPP ADULTS
11 CANDACIA ARMATA COPEPODITES
12 METRIDIA LUCENS COPEPODITES
13 CENTROPAGES TYPICUS ADULTS
14 CALANUS SPP COPEPODITES
15 HYPERIA SPP ADULTS & JUVENILES
16 METRIDIA LUCENS ADULTS
17 CANDACIA ARMATA ADULTS
18 CALANUS FINMARCHICUS ADULTS
19 MEGANYCTIPHANES NORVEGICA ADULT & FURCILIA
20 TOMOPTERIS SPP ADULTS & JUVENILES
21 SAGITTA SPP ADULTS & JUVENILES
0 LESS THAN ONE

FIG. 5. Percentage occurrence of zooplankton taxa in 0.253- and 0.333-mm mesh samples and in the alimentary tracts of larval herring from combined 0.253- and 0.333-mm mesh samples. Taxon numbers increase with increasing length.

5) The extrusion rate of smaller zooplankton taxa was lower at 1.5 knots.

The retention of small larvae is as important to the effective calculation of spawning biomass as the capture of larger larvae is to the prediction of recruitment. Thus, sampling design must be formulated relative to sampling objectives. We feel, however, that higher tow speeds introduce substantial bias to our comprehension and assessment of the dynamics and structure of zooplankton communities. The significantly lower extrusion rates of the smaller zooplankton taxa and the higher feeding incidence of larval herring provide evidence that a more representative portrayal of feeding incidence and selectivity is obtained from samples collected at lower towing speeds. In addition, the quantitative sampling of these smaller forage organisms requires an appreciable reduction in mesh size. But, with a decrease in mesh size there is a corresponding decrease in filtration efficiency associated with increased clogging of the meshes. This places a lower limit to the mesh size that can be used for quantitative sampling (i.e. for zooplankton biomass and production estimates). As long as the flow meter continues to function, the readings should reflect the decrease in the volume of water filtered, so that moderate clogging of the meshes would not seriously affect abundance estimates. Our experience in the Gulf of Maine – Georges Bank area suggests that the 0.165-mm mesh net could be used effectively. In less productive areas, it should be feasible to use a 0.053-mm mesh net for zooplankton abundance estimates. For qualitative sampling of zooplankton (i.e. estimates of the relative numbers of zooplankton in the environment for determining larval food selectivity indices), the 0.053-mm mesh net would be acceptable in all areas. Recent observations (e.g. Lasker 1975; Ellertsen et al. 1979) have demonstrated the efficacy

of using submersible pumps in conjunction with electronic particle counters for in situ measurements of the microdistribution of early stage larval fishes and their planktonic prey organisms. Although these techniques allow for more accurate measures of prey densities than nets towed through the water column, they are subject to bias due to avoidance by larger and more agile plankton organisms and are not in their present stage of development, adaptable to macroscale ichthyoplankton surveys.

While it appears that the herring population and some of the associated invertebrate forage organisms present at the time of this study were adequately sampled at lower towing speeds and with the 0.253-mm mesh net, it is possible that a more mature population of larvae would be better able to avoid nets towed at 1.5 knots and the larger forage organisms utilized by these larger larvae would be more readily retained by a coarser mesh net.

The samples from the 0.333-mm mesh net are cleaner and contain less extraneous material than the 0.253-mm mesh samples and thus are more easily and rapidly sorted for ichthyoplankton. For smaller larval fish taxa (e.g. Gadidae, Labridae, Bothidae, Pleuronectidae) and in surveys designed to estimate spawning biomass and determine spawning times and areas, however, it may be necessary to use finer mesh to ensure complete retention of early stage larvae. Further retention experiments in other areas and at other seasons are required to determine retention rates for these taxa. In future mesh retention studies it would be advisable to use a drogue as a sampling reference point to remain in a specific water mass and population of larvae and to alternate towing speeds consecutively so that the effect of tow speed on extrusion rate could be more accurately determined. Based on our findings from this study, we feel that the most effective broad-scale sampling system would be to use a slow towing speed and a range of mesh sizes chosen for specific taxa and to optimize the efficiency of sampling and sample processing.

The above procedures will not eliminate biases caused by escapement or extrusion and avoidance, but hopefully will minimize some of the technical sources of sampling variability.

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AHLSTROM, E. H., AND J. R. THRAILKILL. 1963. Plankton volume loss with time of preservation. Calif. Coop. Oceanic Fish. Invest. Rep. 9: 57–73.

BUMPUS, D. F., AND L. M. LAUZIER. 1965. Surface circulation on the continental shelf of Eastern North America between Newfoundland and Florida. Am. Geogr. Soc., Serial Atlas of the Marine Environment, Folio 7. 4 p.

ELLERTSEN, B., P. SOLEMDAL, S. SUNDBY, S. TILSETH, T. WESTGÅRD, AND J. ØIESTAD. 1980. Feeding and vertical distribution of cod larvae in relation to availability of prey organisms. Proceedings of ICES Early Life History of Fish Symposium, April 1979. Rapp. P.-V. Reun. Cons. Int. Explor. Mer. (In press)

FAGER, E. W., AND J. A. MCGOWAN. 1963. Zooplankton species groups in the North Pacific. Science 140: 453–460.

HARDY, H. C. 1924. The herring in relation to its animate environment. Part I: the herring with special reference to the east coast of England. Fish. Invest., Long., Ser. II, 7(3): 53 p.

HOLLANDER, M., AND D. A. WOLFE. 1973. Nonparametric statistical methods. John Wiley and Sons, New York, N.Y. 505 p.

ISAACS, J. D., A. FLEMINGER, AND J. K. MILLER. 1969. Distributional atlas of zooplankton biomass in the California Current Region. Spring and Fall 1955–1959. CalCOFI Atlas No. 10: V–XXI, 252 p.

LASKER, R. 1975. Field criteria for survival of anchovy larvae. The relation between inshore chlorophyll maximum layers and successful first feeding. Fish. Bull. (U.S.) 73: 453–462.

LENARZ, W. H. 1972. Mesh retention of *Sardinops caerulea* and *Engraulis mordax* by plankton nets. Fish. Bull. (U.S.) 70: 839–848.

MCEWAN, C. F., M. W. JOHNSON, AND T. R. FOLSOM. 1954. A statistical analysis of the performance of the Folsom plankton sample splitter, based upon test observations. Arch. Meteorol. Geophys. Bioklimatol. Ser. A, 7: 502–527.

SAVILLE, A. 1958. Mesh selection of plankton nets. J. Cons. Perm. Int. Explor. Mer 23: 192–201.

SMITH, P. E., R. C. COUNTS, AND R. I. CLUTTER. 1968. Changes in filtering efficiency of plankton nets due to clogging under tow. J. Cons. Perm. Int. Explor. Mer 32: 232–248.

SMITH, P. E., AND S. L. RICHARDSON. 1977. Standard techniques for pelagic fish egg and larva surveys. FAO Fish. Tech. Pap. 175: 100 p.

SNEDECOR, G. W., AND W. G. COCHRAN. 1967. Statistical methods. 6th ed. Iowa State University Press, Ames, Iowa. 593 p.

TRANTER, D. J., AND P. E. SMITH. 1968. Filtration performance, p. 27–56. *In* D. J. Tranter [ed.] Reviews on zooplankton sampling methods, Part I. UNESCO Monogr. Oceanogr. Methodol. 2, Zooplankton Sampling.

VANNUCCI, M. 1968. Loss of organisms through the meshes, p. 77–86. *In* D. J. Tranter [ed.] Reviews on zooplankton sampling methods, Part I. UNESCO Monogr. Oceanogr. Methodol. 2, Zooplankton Sampling.

WOOD, R. J. 1972. On the loss of herring larvae through nylon nets in a standard high-speed plankton sampler. ICES C.M. 1972/H:14, 10 p. (Mimeo.)

Seston Dynamics in Southern Appalachian Streams: Effects of Clear-cutting

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GURTZ, M. E., J. R. WEBSTER, AND J. B. WALLACE. 1980. Seston dynamics in southern Appalachian streams: effects of clear-cutting. Can. J. Fish. Aquat. Sci. 37: 624–631.

Suspended particulate matter (seston) was studied from July 1977 to July 1978 in two second-order streams in the southern Appalachian Mountains. In the first stream, which drains an undisturbed hardwood forest watershed, seston concentrations fluctuated with season (lowest during winter high flows) and with storm flows. Most organic and inorganic particles were smaller than 105 μm diameter. The second stream drains a watershed (formerly a hardwood forest) that was clear-cut in early 1977. Increased levels of both organic and inorganic seston were found in the latter stream, especially beginning 1 yr after clear-cutting (2 yr after construction of logging roads). Particles larger than 234 μm in diameter accounted for most of the increases in inorganic seston. These increases were probably due to sediments deposited in the stream bed during road building and transported downstream during periods of peak flow. Increased levels of organic seston were probably related to breakdown of debris that entered the stream during logging and reduced retention by leaf packs. We hypothesize that eventual recovery of the stream will be limited by the rate of recovery of the surrounding terrestrial ecosystem.

Key words: seston, sediment, detritus, stream, clear-cut, roads

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Une étude a été faite de la matière particulaire en suspension (seston) dans deux cours d'eau de second ordre du sud des Appalaches de juillet 1977 à juillet 1978. Dans le premier, qui draine un bassin recouvert d'une forêt non dérangée d'arbres feuillus, les concentrations de seston varient avec la saison (minimales au moment de l'abondant débit hivernal) et selon les débits de tempête. La plupart des particules organiques et inorganiques ont un diamètre inférieur à 105 μm. Le second cours d'eau draine un bassin (anciennement forêt d'arbres feuillus) ayant subi une coupe à blanc au début de 1977. On a trouvé dans ce dernier cours d'eau des niveaux plus élevés de seston, tant organique qu'inorganique, surtout à commencer un an après la coupe à blanc (deux ans après la construction de routes forestières). Des particules de diamètre dépassant 234 μm sont responsables de la plupart des augmentations de seston inorganique. Ces augmentations résultent probablement de sédiments déposés sur le lit du cours d'eau lors de la construction de routes et transportés en aval durant les périodes de débit de pointe. Les niveaux accrus de seston organique sont probablement liés à la décomposition des débris pénétrant dans le cours d'eau lors de l'exploitation forestière et à la rétention moindre des amas de feuilles. Nous émettons l'hypothèse que le rétablissement éventuel du cours d'eau sera limité par le taux de rétablissement de l'écosystème terrestre environnant.

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ORGANIC seston in small headwater streams in forested watersheds originates predominantly as allochthonous material (Cummins 1975; Hynes 1975) which, through detrital processing, is successively reduced in size to smaller particles (Boling et al. 1975). Inorganic seston is comprised of particles dislodged from the stream bed or eroded from the stream bank and surrounding watershed. Increased inorganic sediment transport associated with logging activities can often be attributed to construction and use of roads (Brown and Krygier 1971; Douglass and Swift 1977). Some methods of log re-

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moval also increase inorganic seston levels (Bormann and Likens 1970; Douglass and Swift 1977; Patric and Aubertin 1977). Alternatives such as cable logging may have less impact on stream sediment loads (Brown and Krygier 1971).

Several studies have examined organic and/or inorganic material transport in streams and rivers (e.g. Nelson and Scott 1962; Maciolek and Tunzi 1968; Berrie 1972; Fisher and Likens 1973; Sedell et al. 1978; Naiman and Sedell 1979). Bilby and Likens (1979) emphasized the complex relationship between seston concentrations and stream discharge, while others (e.g. Bormann et al. 1974) examined effects of deforestation on particulate transport.

Most seston studies to date have restricted their emphasis to two size fractions, coarse (>1 mm in diameter) and fine (0.45 μm to 1 mm) and ignored the distribution of intermediate sized particles. Particle size distribution may reflect fundamental stream ecosystem processes, including changes predicted by the river continuum hypotheses (Vannote et al. 1980), the role of leaf packs and debris dams as retention devices (Maciolek 1966; Fisher and Likens 1973; Sedell et al. 1978), and the role of detritivores in particle size reduction. Particle size distribution is especially significant to filter feeders, some of which possess fairly size-specific particle capture mechanisms (Wallace and Merritt 1980).

Our research concerned effects of perturbations associated with watershed clear-cutting on seston transport and particle size distribution. We studied two southern Appalachian streams, one that drained an undisturbed watershed and another that drained a watershed which had been recently clear-cut.

Description of Study Area

This study was conducted on two watersheds at Coweeta Hydrologic Laboratory, Franklin, North Carolina. Watershed (WS) 14, drained by Hugh White Creek, is a 61.1-ha (1 ha = 10 000 m^2) watershed with mixed hardwood vegetation that has been undisturbed since 1924 except for the chestnut blight in the early 1930's. Watershed 7, drained by Big Hurricane Branch, is 58.7 ha and is separated from WS 14 by a distance of about 1.4 km. Watershed 7 was clear-cut in 1977. Preclear-cut vegetation was similar to that of WS 14. Preclear-cut characteristics of both watersheds are summarized in Table 1.

Big Hurricane Branch and Hugh White Creek are second-order streams. Each stream is equipped with a V-notch weir for continuous recording of discharge. The hydrograph for Big Hurricane Branch during the study period is shown in Fig. 1. The hydrograph for Hugh White Creek was similar although slightly lower, as discharge in Big Hurricane Branch increased following clear-cutting. Average flow during the 13-mo study period was slightly less than the long-term average; maximum flow during the study period had a recurrence interval of about 2.5 yr.

TABLE 1. Physical and chemical characteristics of the study streams and their watersheds.

	WS 7 Big Hurricane Branch	WS 14 Hugh White Creek
Watershed area (ha)	58.7	61.1
Max. elevation of WS (m)	1060	996
Min. elevation of WS (m)	724	708
Main channel length (m)	1225	1077
Avg. bank-full width[a] (cm)	256	406
Avg. stream depth[a] (cm)	10.5	6.4
Main channel gradient (m·m^{-1})	0.191	0.161
Drainage density	0.0069	—
Avg. annual discharge (L·s^{-1})	17.7[b]	19.0[c]
Avg. annual discharge during study period (L·s^{-1})	22.0	16.8
Max. discharge during study period (L·s^{-1})	363.1	464.4
Min. discharge during study period (L·s^{-1})	9.6	4.0
Avg. annual elemental concentrations (mg·L^{-1})[d]		
NO_3-N	0.002	0.004
NH_4-N	0.004	0.004
PO_4-P	0.002	0.002
Cl	0.699	0.540
SO_4	0.475	0.362
K	0.492	0.350
Na	0.946	0.739
Ca	0.846	0.460
Mg	0.372	0.280
pH	6.82	6.61

[a]Data from first 500 m above weir.
[b]Based on 29 yr of record. All hydrologic data from Coweeta Hydrol. Lab.
[c]Based on 40 yr of record.
[d]Data from Swank and Douglass 1977; WS 7 data is preclear-cut.

As with other headwater streams in the Coweeta Basin, stream gradients of Hugh White Creek and Big Hurricane Branch vary from sections of steep exposed bedrock to short sandy reaches of low-gradient and infrequent small pools. Dense streamside vegetation (mainly rhododendron) provides heavy shading, especially in summer. Levels of autochthonous primary production in undisturbed streams are typically low (J. Hains, Clemson University, Clemson, S.C., August 1978, personal communication), and stream invertebrate communities are dominated by detritivores.

During April–June 1976 three roads were built on WS 7 for logging access. Two of these roads crossed the main stream. Approximately 5% of the watershed area was disturbed by road building. Logging began in January 1977 and was completed in June 1977. A mobile cable system was used for most logging; however, tractor skidding was used on more gentle slopes. Mineral soil was exposed on less than 10% of the total watershed area. Following logging, most of the logging

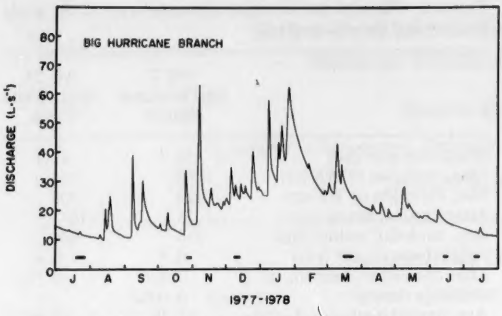

FIG. 1. Hydrograph of Big Hurricane Branch during the study period. Bars indicate times of sample collection. Data from Coweeta Hydrologic Laboratory.

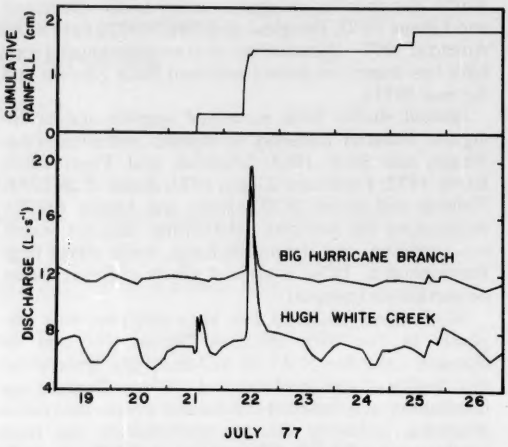

FIG. 2. Rainfall and discharge in the Big Hurricane and Hugh White Creek during the July 1977 sampling period.

debris that fell in or over the stream was removed from the channel.

Methods and Materials

A notched cutoff wall was installed in each stream for sample collection. For known time intervals, drift nets (mesh opening, 230 μm) were placed in these notches so that they caught 100% of the stream flow. These net samples were used for analysis of large particle fractions (>234 μm). Carboys of water were collected for analysis of fine particle fractions (<234 μm).

Samples were analyzed with a wet filtration system. Measured volumes of water or resuspended net samples were filtered with suction through a series of stainless steel sieves of the following size-classes: Coarse (C), greater than 5.0 mm; Large (L), 0.864–5.0 mm; Medium Large (ML), 234–864 μm; Small (S), 105–234 μm; Fine (F), 43–105 μm; and Very Fine (VF), 25–43 μm. Material collected on the screens was resuspended and collected on preashed, preweighed Gelman A/E glass fiber filters. An aliquot of material passing through the VF screen was filtered through a glass fiber filter to provide the Ultrafine (UF) fraction (0.5–25 μm). All samples were oven-dried (50°C, 24 h), desiccated (24 h), weighed, ashed (500°C, 15 min), rewetted (to restore water of hydration; Weber 1973), redried, desiccated, and weighed. From these weights, organic seston was determined as ash-free dry weight and inorganic seston as ash.

Samples were collected intensively on a seasonal basis, during both storm and nonstorm conditions. The first set of samples was collected during eight consecutive days in July 1977, ~1 mo after completion of timber removal from WS 7, and 14 mo after completion of road building. Samples were collected each 6 h initially, more frequently during storms, and less frequently after the stream had returned to base flow. Similar series of samples were collected in October and December 1977, and March, June, and July 1978 (Fig. 1).

Results and Discussion

Effects of Storms

Although storms occurred during four of the six sampling periods, effects of storms on particulate concentrations are perhaps best exemplified by a short-duration, high-intensity storm during July 1977 (Fig. 2). During the first 2 d of the sampling period, a diel flow fluctuation due to evapotranspiration was observed in the undisturbed stream, Hugh White Creek, but diel fluctuation in Big Hurricane Branch was much less pronounced. A short rainfall on the 3rd d occurred on WS 14 but not on WS 7. On the 4th d an intense, short-duration rainfall occurred on both watersheds, followed by a smaller storm on the 7th d. Streamflows increased during storms, but rapidly returned to base flow following each storm. Organic and inorganic particulate material concentrations in Big Hurricane Branch were substantially higher than in Hugh White Creek (Fig. 3). During storms, particulate concentrations increased in both streams. During the storm on July 22, peak organic and inorganic particulate concentrations were 66.8 and 138.3 $mg \cdot L^{-1}$, respectively, in Big Hurricane Branch, and 21.6 and 34.5 $mg \cdot L^{-1}$, respectively, in Hugh White Creek. Following storms, particulate concentrations dropped rapidly to prestorm levels. Particle size distributions did not correspond in a consistent way to changes in discharge.

During the July 1977 storm, peak seston concentrations were reached apparently simultaneously with peak discharge. During this and other storms, a hysteresis effect was observed that shows higher seston concentrations for a given discharge on the rising limb of the hydrograph than for the same discharge on the falling limb (Fig. 4). The shape of this hysteresis loop, however, varies among the storms that occurred during sampling periods. Only once, March 1978, in Hugh White Creek, were seston dynamics similar to those described by Bilby and Likens (1979) — that is peak seston concentrations occurring prior to peak discharge. Paustian and Beschta (1979) found peak suspended

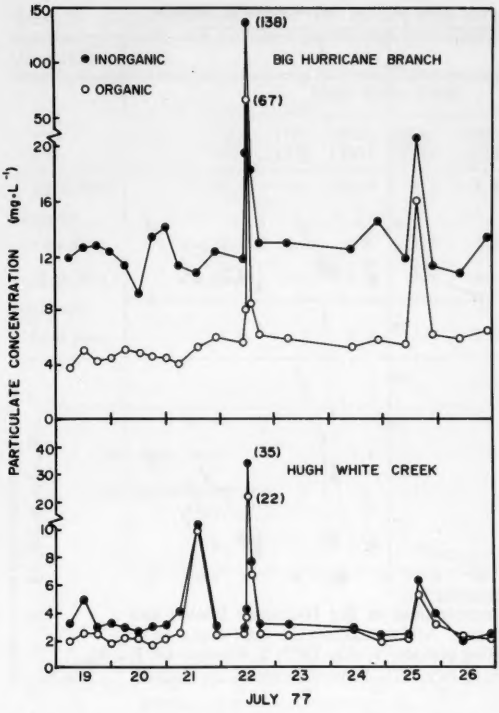

FIG. 3. Organic and inorganic particulate concentrations in Big Hurricane Branch and Hugh White Creek during the July 1977 sampling period. Note change of scale of vertical axes; numbers in parentheses indicate values of points on upper part of axes.

sediment concentrations during a given storm occurring before or at the hydrograph peak, the pattern apparently dependent upon the shape of the hydrograph prior to the storm. Bilby and Likens (1979) also noted that seston concentrations were lower after discharge returned to prestorm levels than before the storm, suggesting a "wash-out" effect. While this effect seems valid for certain sets of conditions, we did not observe it.

SEASONAL CHANGES IN SESTON CONCENTRATIONS

Mean concentrations of total inorganic and organic seston for nonstorm samples taken during each sampling period are presented in Table 2. In Hugh White Creek, high inorganic and organic seston concentrations were observed in summer whereas low concentrations occurred in winter. Factors possibly contributing to this seasonal pattern include dilution during high winter base flow; entrapment of fine particulate material in leaf packs during late fall and winter; reduced winter processing rates because of lower temperatures and insufficient leaf conditioning; and a long-term washout effect following early fall storms. Bormann et al. (1969, 1974) also found lower winter seston transport rates in smaller headwater mountain streams draining deciduous forest watersheds in northeastern United States. However, Naiman and Sedell (1979) found highest organic seston transport during winter in low-order streams in Oregon. Wetzel and Manny (1977) found highest organic seston concentrations in summer in a first-order stream draining a marsh in Michigan.

Seasonal seston dynamics in Big Hurricane Branch differed from the pattern we observed in the undisturbed stream. Seston concentrations in Big Hurricane Branch were consistently higher than in Hugh White Creek, and the magnitude of difference increased toward the end of our study. While seston concentrations decreased in December, the effect of clear-cutting on seasonal seston dynamics was partially masked by the longer term trend of increasing transport (Table 2 and 3).

RELATIONSHIP BETWEEN INORGANIC AND ORGANIC MATERIAL

We found a consistent relationship between inorganic and organic material in Hugh White Creek (Fig. 5; $r = 0.96$, $n = 68$). Inorganic material averaged 57.5% of the particulate load throughout the study. A similar relationship existed in Big Hurricane Branch prior to the March 1978 samples. However, in the later (March, June, July 1978) samples, the inorganic fraction increased without a similar increase in organic material.

PARTICLE SIZE DISTRIBUTION

Distribution of inorganic and organic seston (nonstorm samples) is presented by size class in Fig. 6. In the undisturbed stream, most particles (by weight) were in the three smallest size classes, i.e. $<105\ \mu m$. This is true for all seasons and for both inorganic and organic material. Concentrations of all size fractions were lowest in winter. Organic particles larger than $234\ \mu m$ (ML and larger) were negligible except in October during leaf fall.

In Big Hurricane Branch, concentrations of both inorganic and organic particles were greater in all size fractions. Beginning in October 1977 (following removal of timber slash from the stream bed), particles larger than $234\ \mu m$ increased proportionately more than smaller particles, especially in the inorganic fraction. Thus, the increase in seston concentrations shown in Table 2 occurred mainly in the ML and L size-classes ($234\ \mu m$–5 mm).

Median particle size (by weight) generally ranged from 35 to $65\ \mu m$ (Fig. 7). Median organic particle size in Big Hurricane Branch was greater than that of Hugh White Creek except for the July 1977 sample. A continuous trend toward larger inorganic particles in suspension was apparent in Big Hurricane Branch,

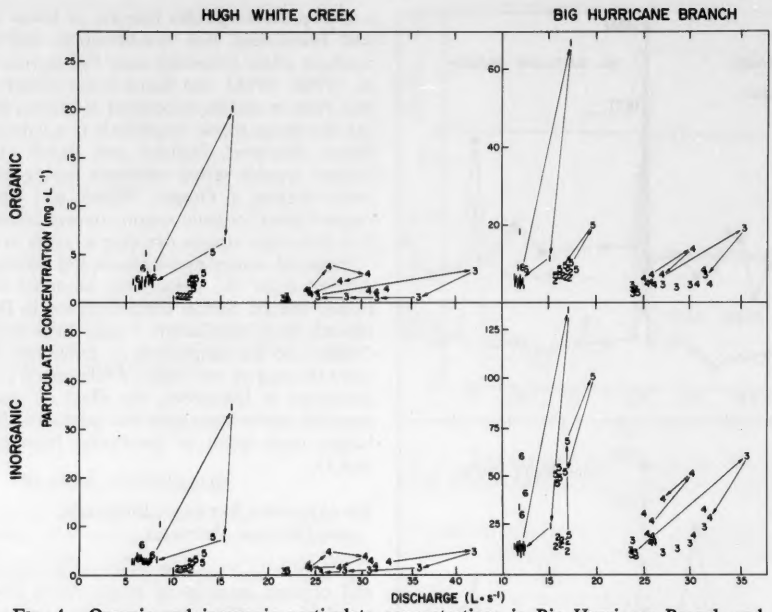

FIG. 4. Organic and inorganic particulate concentrations in Big Hurricane Branch and Hugh White Creek as functions of stream discharge. Arrows indicate the time sequence of storm samples. Numbers indicate various sampling periods: 1, July 1977; 2, October 1977; 3, December 1977; 4, March 1978; 5, June 1978; 6, July 1978. Some data points are masked.

increasing dramatically in June 1978 (Fig. 7).

Particle size of organic material in Hugh White Creek was generally larger than reported for other low-order streams by Sedell et al. (1978). Median particle size in Hugh White Creek ranged from 37 to 65 μm, whereas Sedell et al. reported median particle sizes between 5 and 12 μm. However, except for winter samples, they did not fractionate material between 53 and 0.45 μm. These figures emphasize the relative importance of small particles in all streams studied and reflect a greater importance of somewhat larger particles in Coweeta streams.

TABLE 2. Mean seston concentrations during low flow (nonstorm) conditions. S_m is standard error of the mean. n is the number of samples.

		Hugh White Creek						Big Hurricane Branch					
		July 1977	Oct. 1977	Dec. 1977	Mar. 1978	June 1978	July 1978	July 1977	Oct. 1977	Dec. 1977	Mar. 1978	June 1978	July 1978
Stream flow ($L \cdot s^{-1}$)		6.8	11.2	22.0	31.6	11.9	6.9	12.1	16.6	24.3	31.4	16.0	12.3
Particulate organic material ($mg \cdot L^{-1}$)	$\bar{x}$	2.11	0.87	0.31	1.29	2.11	3.01	4.54	6.98	2.64	6.43	7.70	8.25
	S_m	0.10	0.07	0.02	0.12	0.14	0.38	0.14	0.32	0.18	0.82	0.51	0.14
	n	9	8	6	5	4	3	9	8	6	5	4	3
Particulate inorganic material ($mg \cdot L^{-1}$)	$\bar{x}$	3.32	1.11	0.42	1.99	2.70	3.99	12.21	15.54	9.68	28.64	49.96	42.61
	S_m	0.24	0.05	0.02	0.29	0.12	0.51	0.47	0.97	0.33	2.92	1.72	9.34
	n	9	8	6	5	4	3	9	8	6	5	4	3

TABLE 3. Seston transported ($kg \cdot d^{-1}$) by the two study streams during low and high flows. Low flow values are means of all nonstorm data from each collection period. High flow values represent data from the highest storm flow which occurred during the sampling period.

	Hugh White Creek						Big Hurricane Branch					
	July 1977	Oct. 1977	Dec. 1977	Mar. 1978	June 1978	July 1978	July 1977	Oct. 1977	Dec. 1977	Mar. 1978	June 1978	July 1978
High flow	30.2	—	10.9	8.2	3.4	—	99.8	—	58.7	35.6	33.2	—
Organic												
Low flow	1.2	0.8	0.6	3.5	2.2	1.8	4.7	10.0	5.5	17.4	10.6	8.8
High flow	48.3	—	16.8	9.5	9.1	—	206.6	—	185.2	137.0	172.6	—
Inorganic												
Low flow	1.9	1.1	0.8	5.4	2.8	2.4	12.8	22.3	20.3	77.7	69.1	45.3

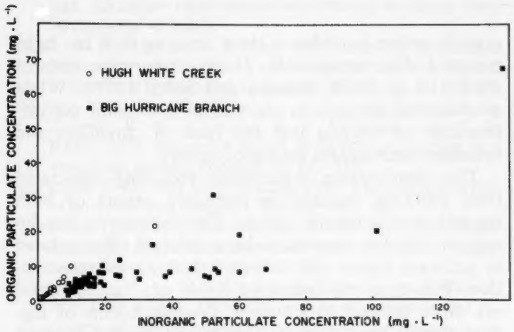
ORGANIC PARTICULATE CONCENTRATION (mg · L⁻¹)
HUGH WHITE CREEK
BIG HURRICANE BRANCH
INORGANIC PARTICULATE CONCENTRATION (mg · L⁻¹)

FIG. 5. Relationship between inorganic and organic seston in Big Hurricane Branch and Hugh White Creek. Some data points are masked.

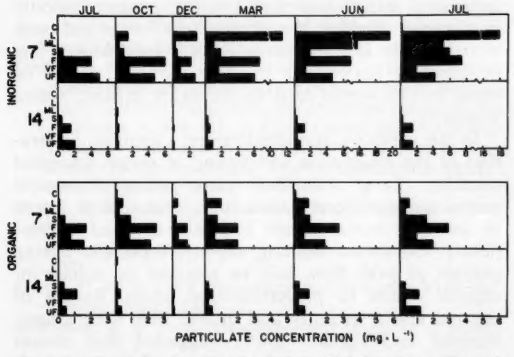

FIG. 6. Particle size distribution in Big Hurricane Branch and Hugh White Creek, July 1977 through 1978. Values are means of nonstorm samples. 14 refers to Hugh White Creek, the undisturbed stream, and 7 to Big Hurricane Branch, which drains the clear-cut watershed. Size-classes are C, >5.0 mm; L, 0.864–5.0 mm; ML, 234–864 μm; S, 105–234 μm; F, 43–105 μm; VF, 25–43 μm; and UF, 0.5–25 μm.

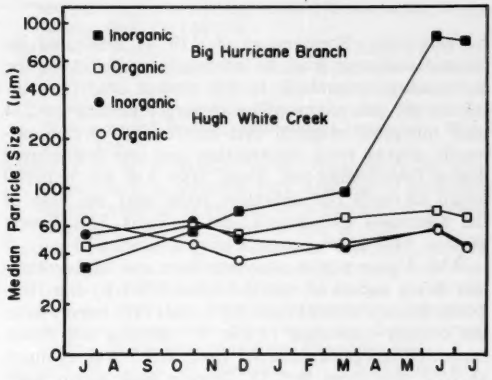

FIG. 7. Median particle sizes, by weight, in Big Hurricane Branch and Hugh White Creek during nonstorm conditions.

AVERAGE SESTON CONCENTRATIONS AND TRANSPORT

Table 4 presents mean annual particulate organic concentrations for low-order streams. Hugh White Creek had levels of organic seston within the range of other unperturbed forest streams. Big Hurricane Branch had an annual mean organic seston load nearly five times as high as Hugh White Creek and greater than most other streams. Streams higher than Big Hurricane Branch include low-gradient brown water Michigan streams and streams that have undergone some changes from their natural condition, e.g. Stampen Stream which flows through some agricultural land and is eutrophic (Malmqvist et al. 1978), and the Rhode River which drains low-gradient, multiple-use watersheds that are less than 50% forested (Pierce and Dulong 1977).

Inorganic seston concentrations in Big Hurricane Branch rose abruptly during the second summer after clear-cutting, a finding similar to that reported by Bormann et al. (1974). At Hubbard Brook, however, there were no roads and regrowth vegetation was inhibited

TABLE 4. Mean annual particulate organic matter (POM) in low-order streams. Stream order in parentheses.

Stream	POM ($mg \cdot L^{-1}$)	Reference
Bear Brook, New Hampshire (2)	0.32	Fisher and Likens (1973)
Mack Cr., Oregon (3)	0.59	Naiman and Sedell (1979)
Convict Cr., California (2)	0.67	Maciolek (1966)
Devils Club Cr., Oregon (1)	0.97	Naiman and Sedell (1979)
Hugh White Cr., North Carolina (2)	1.14	This study
White Clay Cr., Pennsylvania (2)	1.36	Sedell et al. (1978)
White Clay Cr., Pennsylvania (3)	1.59	Sedell et al. (1978)
Camp Creek, Idaho (2)	2.62	Sedell et al. (1978)
Big Hurricane Branch, North Carolina (2)	5.15	This study
Smith Cr., Michigan (1)	5.89	Sedell et al. (1978)
Augusta Cr., Michigan (2)	7.13	Sedell et al. (1978)
Stampen Stream, Sweden (2)	4–22	Malmqvist et al. (1978)
Rhode R. tributaries, Maryland (2)	3.7–19.4	Pierce and Dulong (1977)

by herbicides. Bormann et al. (1974) attributed increased sediment load to increased erodibility in the surrounding watershed. In the present study, we attribute the delayed response to larger particle (>234 μm) inorganic material that entered the stream primarily during road construction and was transported during flood conditions. Then, after 2 yr this material began to reach our collection point near the base of the watershed (W. Swank and L. Swift, U.S. Forest Service, May 1979, personal communication).

With higher seston concentrations and higher summer flows, export of material from WS 7 by Big Hurricane Branch was substantially higher than export from the control watershed (Table 3). During low flows, export of inorganic material from WS 7 was as much as 25× that from WS 14. During peak storm flows the greatest difference we found was 19× that of WS 14. Export of organic material was also higher in Big Hurricane Branch, as much as 11× during nonstorm flows and 10× higher during storms.

Conclusions

The importance of small particles in seston of flowing waters has only recently received attention (Sedell et al. 1978; Naiman and Sedell 1979). Our studies showed that an undisturbed headwater stream in the southern Appalachians carries in suspension particles that are mostly less than 105 μm in diameter. For the organic fraction, this indicates that allochthonous inputs are comminuted within the upper reaches of the stream through physical and microbial processes and detritivore feeding. Large particle detritivores (shredders) feeding on coarse material generate particles mainly in the ML range (234–864 μm) (J. O'Hop, Univ. of Georgia, GA, May 1979, personal communication). Further breakdown occurs as this material settles out or becomes trapped and is ingested by other organisms, consistent with the "spiralling" concept (Webster 1975; Wallace et al. 1977).

We found a greater relative abundance of larger organic seston particles in these streams than has been reported for comparable North American streams (Sedell et al. 1978; Naiman and Sedell 1979). While available information is insufficient to explain the differences, we suggest that the roles of shredders and retention mechanisms are major factors.

The clear-cutting experiment, including associated road building, resulted in increased export of both organic and inorganic seston. The increase in the inorganic fraction seemed to be a delayed effect related to sediment inputs that occurred during road construction. However, the increased levels of organic seston are more difficult to interpret. Possible effects of logging debris input and its subsequent partial removal from the stream confound interpretation. Debris in the stream represents both a source of seston and a mechanism for seston retention (Naiman and Sedell 1979). Thus, breakdown of logging debris in some channel areas may lead to increased levels of organic material in transport. Further, the observed decline in leaf pack abundance in Big Hurricane Branch represents a loss in retention devices for trapping particles. Either of these factors would lead to increased organic seston transport.

In this paper we have documented a significant alteration of the magnitude and timing of seston transport resulting from watershed clear-cutting. Inorganic seston concentrations remain high, even though inputs of sediment to the stream bed have declined tremendously. Continued flushing activity, especially during periods of peak flow, will be required to reduce inorganic seston to preperturbation levels. Return of organic seston quantity and quality may take longer. Webster and Patten (1979) suggested that stream ecosystems have low resistance to perturbation but high resilience following perturbation. However, a major aspect of stream resilience is replenishment of biomass through allochthonous inputs. Recovery of allochthonous input (quantity, quality, and timing) to preperturbation levels depends on the resilience of the forest ecosystem, not the stream. Hence, recovery of

streams from watershed perturbation is limited by the rate of recovery of the surrounding terrestrial ecosystem.

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BERRIE, A. D. 1972. The occurrence and composition of seston in the River Thames and the role of detritus as an energy source for secondary production in the river. Mem. Ist. Ital. Idrobiol. Dott Marco de Marchi Pallanza Italy 29 (Suppl.): 473–483.

BILBY, R. E., AND G. E. LIKENS. 1979. Effect of hydrologic fluctuations on the transport of fine particulate organic carbon in a small stream. Limnol. Oceanogr. 24: 69–75.

BOLING, R. H. JR., D. GOODMAN, J. A. VAN SICKLE, J. O. ZIMMER, K. W. CUMMINS, R. C. PETERSEN, AND S. R. REICE. 1975. Toward a model of detritus processing in a woodland stream. Ecology 56: 141–151.

BORMANN, F. H., AND G. E. LIKENS. 1970. The nutrient cycles of an ecosystem. Sci. Am. 223: 92–101.

BORMANN, F. H., G. E. LIKENS, AND J. S. EATON. 1969. Biotic regulation of particulate and solution losses from a forest ecosystem. BioScience 19: 600–610

BORMANN, F. H., G. E. LIKENS, T. G. SICCAMA, R. S. PIERCE, AND J. S. EATON. 1974. The export of nutrients and recovery of stable conditions following deforestation at Hubbard Brook. Ecol. Monogr. 44: 255–277.

BROWN, G. W., AND J. T. KRYGIER. 1971. Clear-cut logging and sediment production in the Oregon Coast Range. Water Resour. Res. 7: 1189–1198.

CUMMINS, K. W. 1975. The ecology of running water—theory and practice, p. 278–293. *In* D. B. Baker, W. B. Jackson, and B. L. Prater [ed.] Proc. of Sandusky River Basin Symposium, Tiffin, Ohio.

DOUGLASS, J. E., AND L. W. SWIFT JR. 1977. Forest Service studies of soil and nutrient losses caused by roads, logging, mechanical site preparation, and prescribed burning in the Southeast, p. 489–503. *In* D. L. Correll [ed.] Watershed research in eastern North America. Chesapeake Bay Center for Environmental Studies, Smithsonian Institution, Edgewater, Md.

FISHER, S. G., AND G. E. LIKENS. 1973. Energy flow in Bear Brook, New Hampshire: an integrative approach to stream ecosystem metabolism. Ecol. Monogr. 43: 421–439.

HYNES, H. B. N. 1975. The stream and its valley. Verh. Int. Verein. Limnol. 19: 1–15.

MACIOLEK, J. A. 1966. Abundance and character of microseston in a California mountain stream. Verh. Int. Verein. Limnol. 16: 639–645.

MACIOLEK, J. A., AND M. G. TUNZI. 1968. Microseston dynamics in a simple Sierra Nevada lake–stream system. Ecology 49: 60–75.

MALMQVIST, B., L. M. NILSSON, AND B. S. SVENSSON. 1978. Dynamics of detritus in a small stream in southern Sweden and its influence on the distribution of the bottom animal communities. Oikos 31: 3–16.

NAIMAN, R. J., AND J. R. SEDELL. 1979. Characterization of particulate organic matter transported in some Cascade Mountain streams. J. Fish. Res. Board Can. 36: 17–31.

NELSON, D. J., AND D. C. SCOTT. 1962. Role of detritus in the productivity of a rock-outcrop community in a Piedmont stream. Limnol. Oceanogr. 7: 396–413.

PATRIC, J. H., AND G. M. AUBERTIN. 1977. Long-term effects of repeated logging on an Appalachian stream. J. For. 75: 492–494.

PAUSTIAN, S. J., AND R. L. BESCHTA. 1979. The suspended sediment regime of an Oregon Coast Range stream. Water Resour. Bull. 15: 144–154.

PIERCE, J. W., AND F. T. DULONG. 1977. Discharge of suspended particulates from Rhode River subwatersheds, p. 531–554. *In* D. L. Correll [ed.] Watershed research in eastern North America. Chesapeake Bay Center for Environmental Studies, Smithsonian Institution, Edgewater, Md.

SEDELL, J. R., R. J. NAIMAN, K. W. CUMMINS, G. W. MINSHALL, AND R. L. VANNOTE. 1978. Transport of particulate organic material in streams as a function of physical processes. Verh. Int. Verein. Limnol. 20: 1366–1375.

SWANK, W. T., AND J. E. DOUGLASS. 1977. Nutrient budgets for undisturbed and manipulated hardwood forest ecosystems in the mountains of North Carolina, p. 342–362. *In* D. L. Correll [ed.] Watershed research in eastern North America. Chesapeake Bay Center for Environmental Studies, Smithsonian Institution, Edgewater, Md.

VANNOTE, R. L., G. W. MINSHALL, K. W. CUMMINS, J. R. SEDELL, AND C. E. CUSHING. 1980. The river continuum concept. Can. J. Fish. Aquat. Sci. 37: 130–137.

WALLACE, J. B., J. R. WEBSTER, AND W. R. WOODALL. 1977. The role of filter feeders in flowing waters. Arch. Hydrobiol. 79: 506–532.

WALLACE, J. B., AND R. N. MERRITT. 1980. Ecology of filter-feeding aquatic insects. Annu. Rev. Entomol. 25: 103–132.

WEBER, C. I. [ed.] 1973. Biological field and laboratory methods for measuring the quality of surface water and effluents. EPA 670/4-73-001, Cincinnati, Ohio.

WEBSTER, J. R. 1975. Analysis of potassium and calcium dynamics in stream ecosystems on three southern Appalachian watersheds of contrasting vegetation. Dissertation, Univ. Georgia., Athens, Ga.

WEBSTER, J. R., AND B. C. PATTEN. 1979. Effects of watershed peturbation on stream potassium and calcium budgets. Ecol. Monogr. 49: 51–72.

WETZEL, R. G., AND B. A. MANNY. 1977. Seasonal changes in particulate and dissolved organic carbon and nitrogen in a hardwood stream. Arch. Hydrobiol. 80: 20–39.

Diets of *Ictalurus nebulosus* and *Pomoxis nigromaculatus* altered by diflubenzuron

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Diflubenzuron was applied to five experimental ponds, yielding a mean concentration of 13 μg/L. Residues in the water declined below detectable limits (0.2 μg/L) by 14 d posttreatment. Young-of-the-year black crappie (*Pomoxis nigromaculatus*) and brown bullhead (*Ictalurus nebulosus*) accumulated diflubenzuron and then eliminated all residues by 7 d posttreatment. No fish mortalities occurred after the treatment. For 1 mo following treatment, stomach content analyses indicated major alterations in the diets of the fish. Growth rates and condition factors of the fish 3 mo after treatment were similar to control fish in four untreated ponds.

Key words: diflubenzuron, *Pomoxis nigromaculatus*, *Ictalurus nebulosus*, condition factor, growth, aquatic invertebrates

COLWELL, A. E., AND C. H. SCHAEFER. 1980. Diets of *Ictalurus nebulosus* and *Pomoxis nigromaculatus* altered by diflubenzuron. Can. J. Fish. Aquat. Sci. 37: 632–639.

A cinq étangs expérimentaux, on appliqua du diflubenzuron pour donner une concentration moyenne de 13 μg/L. Quatorze jours après traitement, les résidus dans l'eau diminuent au-dessous des limites décelables (0,2 μg/L). Les jeunes de l'année de marigane noire (*Pomoxis nigromaculatus*) et barbotte brune (*Ictalurus nebulosus*) accumulent le diflubenzuron et ensuite, 7 d après traitement, éliminent tous les résidus. Aucune mortalité ne se produit après traitement. Pendant 1 mois après traitement, des analyses de contenus stomacaux indiquent des changements majeures de régime alimentaire des poissons. Les taux de croissance et les facteurs de condition 3 mois après traitement sont semblables à ceux de témoins maintenus dans quatre étangs non traités.

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DIFLUBENZURON [1-(4-chlorophenyl)-3-(2, 6 difluorobenzoyl)-urea] is an insect growth regulator which inhibits the synthesis of chitin (Mulder and Gijwijt 1973; Post and Vincent 1973). As a larvicide, it is effective in controlling populations of some pestiferous dipterans (Schaefer and Wilder 1973; Mulla et al. 1975; Steelman et al. 1975; Apperson et al. 1978; Rodriguez and Wright 1978). It would not be expected to cause direct fish mortality unless some species or age classes greatly accumulated this compound (Julin and Sanders 1978). However, some reports suggest that diflubenzuron may adversely affect some species of nontarget aquatic invertebrates (Miura and Takahashi 1975; Ali and Mulla 1978).

In the present study diflubenzuron residues in black crappie (*Pomoxis nigromaculatus*) and brown bullhead (*Ictalurus nebulosus*) were determined for evaluation of the accumulation potential of diflubenzuron under field treatment conditions. The study was also designed to determine effects of diflubenzuron on invertebrate prey organisms, and to correlate these findings with effects on fish.

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Materials and Methods

The study was conducted in 1977 at nine experimental ponds in Lakeport, California. Each rectangular pond had a surface area of about 0.01 ha (1 ha = 10 000 m²) and a depth of 1.2 m. Every pond had a float valve which maintained the water level with water from a well. The pH of the nine ponds ranged from 6.8 to 8. 1 (mean = 7.7) at the time of the treatment and decreased somewhat (minimum recorded pH = 6.5) during the study. Electrical conductivity values in the ponds were typically 730–860 μmhos/cm. Midwater temperatures in the ponds were normally 21–25°C.

A portion of a pooled sample containing plankton from all the ponds was introduced into each pond so that every pond was inoculated with similar species of plankton on June 30 and again on July 10. On July 14, *I. nebulosus* were obtained with a cast net from Clear Lake, California, and 600 similarly sized young-of-the-year fish were placed

in each of ponds 5–9. Ponds 1–4 were stocked on August 3 with young-of-the-year *P. nigromaculatus* (295 fish per pond) which had been seined from Lake Mendocino, California.

Water samples for diflubenzuron residue analyses were taken with a 3-L Van Dorn bottle at top, middle, and bottom depths in two locations from each pond 1 h prior to treatment and 1, 5, 24, 72, 168, and 336 h after the treatment; 600 mL of each sample were partitioned against 200 mL of freshly distilled methylene chloride. In the laboratory, the aqueous fraction was again partitioned against 2×200 mL of methylene chloride within 48 h of the time of collection, and the combined fractions were analyzed by high pressure liquid chromatography (HPLC) (Schaefer and Dupras 1977).

Zooplankton was sampled by taking a vertical tow with a No. 12 standard plankton net at three locations in each pond at 12, 9, 7, 5, and 2 d and at 2 h before treatment; and at 1, 2, 5, 8, 15, 22, 29, 37, 43, 50, 57, 65, 72, and 78 d posttreatment. The samples were preserved in a 4% formaldehyde solution containing rose bengal dye. In the laboratory, the sample was mixed, a 5% subsample was removed, and the organisms were identified (Edmondson 1963; Usinger 1956) and enumerated. Cladoceran, copepod, and rotifer data were analyzed using chi-square analyses of pond populations increasing vs. decreasing from pretreatment levels.

Benthos was monitored by taking three standard Ekman dredge samples (each covering an area of about 232 cm^2) from each pond at 14, 12, 9, 7, 5, and 2 d and at 1 h before treatment; and 2, 5, 8, 15, 22, 29, 37, 43, 51, 58, 65, 72, and 77 d following treatment. Samples were analyzed according to the methods of Snell and Hazeltine (1963). Significance of difference between means for control and treated ponds in the density of benthic organisms was determined by Student's *t*-test for each sampling date.

Fish for residue analyses were removed from the ponds by seining, placed in labeled polyethylene bags, and immediately packed in ice in the field at 1 d prior to treatment and at 1, 2, 4, and 7 d after treatment. In the laboratory the fish were sealed in 0.95-L tin cans and frozen within 2 h of the time of collection. Later, at least 10 fish per pond per sampling date were analyzed for diflubenzuron residues by HPLC according to the methods of Diprima et al. (1978). At least once per week, each pond was examined for fish mortality. All fish floating on the water surface were counted and removed.

For stomach content analyses and growth studies, 10 *I. nebulosus* were seined from each pond (5–9) between 08:00 and 10:00 at 5 and 1 d before treatment; and at 1, 2, 4, 7, 29, 36, and 79 d following treatment. Ten *P. nigromaculatus* were seined from each remaining pond between 10:00 and 11:30 at 5 and 1 d before treatment; and at 1, 2, 4, 7, 14, 21, 29, 36, and 79 d following treatment. Moisture was blotted from each fish and the fish fresh weight and standard length were determined. Each fish was placed in a glass vial bearing the fish identification number and collection date. The vial was then sealed with a plastic lid and stored at −10°C. Later, each fish was removed from the freezer, its stomach was dissected, and the stomach contents were weighed and then washed with distilled water into a counting dish. The fish carcass was dried at 60°C for 72 h and weighed. Percentage dry weight of the fish soma (carcass without stomach contents) was then calculated.

The organisms in the fish stomach were identified and enumerated. The stomach contents were then placed in a filter tube, the water volume was brought up to 25 mL, and this sample was then strained under partial vacuum through a 2.4-cm Whatman GF/A glass fibre filter which had previously been washed with distilled water, dried, and weighed. The stomach content filter was dried at 60°C for 24 h. The sample was then weighed and the percentage dry weight of the stomach contents was calculated. Stomach content and percentage dry weight of fish from control and treated ponds were compared using Student's *t*-tests.

Results and Discussion

Pretreatment water samples indicated that there was no detectable diflubenzuron in any of the ponds prior to treatment. In each of the treated ponds, the highest diflubenzuron concentration occurred in a top water sample (Table 1). At 1 h after treatment, the treated ponds had a mean concentration of 13.2 μg/L. The residues decreased rapidly with time and were below detectable limits in most of the ponds by 14 d (336 h) posttreatment.

The most abundant cladocerans in the zooplankton samples were *Ceriodaphnia* sp., *Diaphanosoma* sp., *Chydorus* sp., *Bosmina* sp., and *Daphnia* sp. All of these cladocerans experienced population reductions in all of the treated ponds within a few days after the diflubenzuron application. In pond 5, the *Ceriodaphnia* sp. recovered rapidly and exceeded pretreatment densities by 1 wk posttreatment, but most cladoceran densities in treated ponds recovered more slowly.

Monitoring of copepod populations indicated that the abundance of nauplii decreased in all ponds following the treatment, and returned to pretreatment densities in from 7 d (pond 2) to >4 wk (pond 8) posttreatment. Among the copepodites and adults, the susceptibilities to diflubenzuron of the *Diaptomus* sp. (filter feeders) and the *Cyclops* sp. (which are often predatory) were similar, although in most of the treated ponds the *Diaptomus* sp. populations experienced more rapid recoveries than the *Cyclops* sp. populations.

The detritivorous rotifers (*Brachionus* sp., *Keratella* sp., and *Hexarthra* sp.) increased in abundance in both the control and treated ponds during the first 8 d following the treatment. Similarly, the *Asplanchna* sp. (a mostly predatory rotifer) mean density in the treated ponds increased from 0.18 organisms per litre pretreatment to 0.43 organisms per litre 8 d posttreatment, while corresponding changes occurred in the *Asplanchna* sp. densities in the control ponds.

The population changes of the zooplankton were similar in the ponds containing *P. nigromaculatus* and in those containing *I. nebulosus*. However, the *Cyclops* sp. populations in the treated *I. nebulosus* ponds experienced greater declines and slower recoveries than the *Cyclops* sp. populations in the treated *P. nigromaculatus* ponds. Another minor difference was that while a high pretreatment *Keratella* sp. level in *P. nigromaculatus* pond 4 was never approached in posttreatment samples, the late posttreatment levels in

TABLE 1. Residues of diflubenzuron in water of experimental ponds after treatment.

Pond	Sample depth	Mean concentration (μg/L) 1 h	5 h	24 h	72 h	168 h	336 h
2	Top	15.8	9.4	6.8	2.2	0.9	0.2
	Middle	8.8	13.6	6.5	2.2	0.4	N.D.[a]
	Bottom	1.7	7.0	6.5	2.6	0.2	0.4
4	Top	22.9	18.1	7.0	1.2	N.D.	N.D.
	Middle	13.8	24.1	7.0	1.2	N.D.	N.D.
	Bottom	0.4	6.8	7.7	1.6	N.D.	N.D.
5	Top	39.1	19.4	7.9	1.4	N.D.	N.D.
	Middle	3.9	9.9	8.7	1.4	0.2	N.D.
	Bottom	1.1	3.6	7.6	1.0	N.D.	N.D.
6	Top	29.4	7.6	8.3	1.1	N.D.	N.D.
	Middle	18.0	13.5	8.3	0.8	N.D.	N.D.
	Bottom	4.8	9.4	7.8	0.8	N.D.	0.2
8	Top	15.6	7.2	6.2	1.4	0.2	N.D.
	Middle	14.6	15.0	5.6	2.6	N.D.	N D.
	Bottom	7.5	13.8	5.5	1.6	0.2	N.D.

[a]N.D. = not detected (<0.2 μg/L).

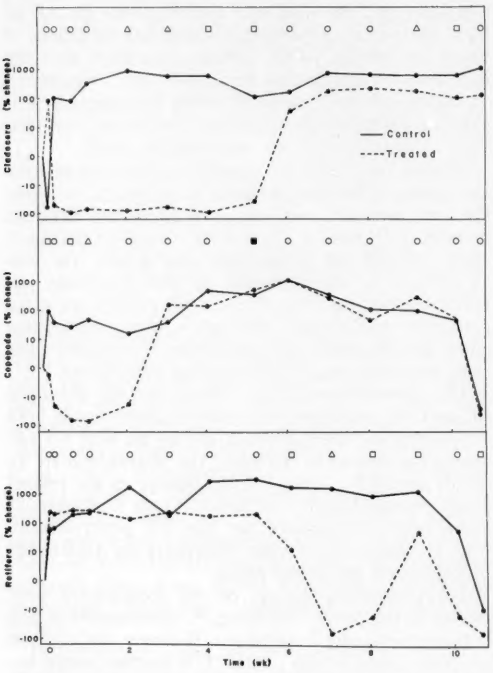

FIG. 1. Percent change from pretreatment densities of organisms in control (1, 3, 7, and 9) and treated (2, 4, 5, 6, and 8) ponds. Symbol indicates significance of difference between control and treated ponds; ○ = $P > 0.10$, □ = $0.10 < P > 0.05$, △ = $0.05 < P > 0.01$. Solid symbols indicate treated value significantly greater than control value.

treated pond 8 (and control pond 7) greatly exceeded any of the pretreatment *Keratella* sp. levels. Some of these differences may be due to natural interpond variation.

To reduce the chance effects of interpond variation, the average percentage of changes from pretreatment values for all the control ponds (1, 3, 7, and 9) and for all the treated ponds (2, 4, 5, 6, and 8) are presented in Fig. 1. During the first 5 d following treatment, the cladocerans in the treated ponds declined by >99% from the pretreatment values. Since some major changes in invertebrate population levels are normal, the importance of this decline in the treated cladocerans becomes more apparent when compared with the 95% increase in the control cladocerans during the same period. The treated cladoceran populations did not start to recover until more than 4 wk posttreatment, and did not return to pretreatment densities until more than 5 wk after treatment.

The reductions of the treated copepods (Fig. 1) were of lesser magnitude and of shorter duration than the cladoceran reductions. Cladocerans molt (and synthesize chitin) in all life stages, whereas copepods do not molt after reaching the adult instar (Barnes 1974). Because some adult copepods can live for >3 wk, they may provide a reservoir group until the water residues decline below lethal limits for the molting stages. If this explanation for the ability of copepods to survive a diflubenzuron treatment were correct, an increase in the average size of the copepods might be expected as mortality of the small molting copepodites occurred. However, the mean length (0.67 mm) for 2–8-d pretreatment *Cyclops* sp. was not significantly different (*t*-test $P > 0.05$) from the mean length (0.68 mm) for the 2–8-d posttreatment *Cyclops* sp. Similarly, the *Diaptomus* sp. mean length (0.49 mm) 2–8 d before

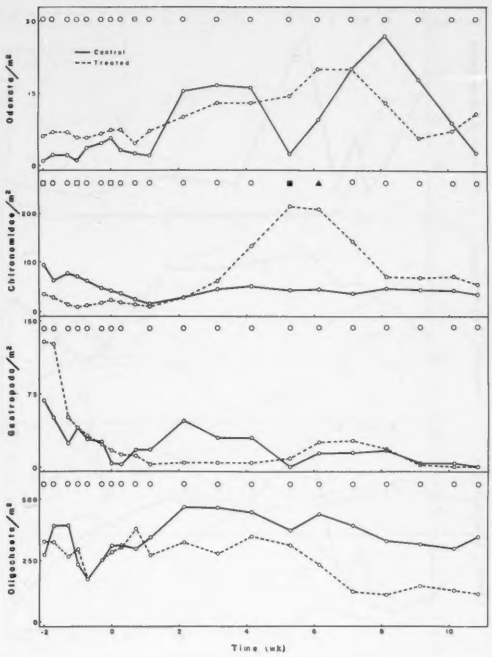

FIG. 2. Mean number of benthic organisms per m^2 in control (1, 3, 7, and 9) and treated (2, 4, 5, 6, and 8) ponds. Data are plotted as 3-point running means. Treatment occurred on day 0. Symbol indicates significance of difference between control and treated values; ○ = $P > 0.10$, □ = $0.10 < P > 0.05$, △ = $0.05 < P > 0.01$, ⬡ = $P < 0.01$. Solid symbol indicates treated value significantly greater than control value.

treatment was not different ($P > 0.05$) from the *Diaptomus* sp. mean length (0.50 mm) 2–8 d posttreatment. Predation on the larger copepods, the renewed development of resting benthic copepodites, or other factors may have obscured possible effects of diflubenzuron on the size frequency distribution of the copepods.

Diflubenzuron did not have any apparent effect on the rotifers (Fig. 1). During the first 2 wk posttreatment when there were detectable levels of diflubenzuron in the ponds, the number of rotifers in the treated ponds increased, but these were apparently normal population changes, since there were no significant differences between the control and the treated ponds.

Figure 2 shows the densities of benthic organisms in the four control ponds and the five treated ponds. Shortly after treatment, the odonate and chironomid densities in the treated ponds declined by 35 and 47%, respectively, and increased again by 14 and 16 d posttreatment, respectively. However, similar changes occurred in the control ponds during this time, so these changes apparently were not due to the diflubenzuron application.

TABLE 2. Residues of diflubenzuron in fish from experimental ponds treated with 13 ng/g.[a] The chemical was not detected (<8.3 ng/g) in fish before treatment.

Pond	Fish	Concentration (ng/g) Days posttreatment 1	2	4	7
2	*P. nigromaculatus*	386.3	183.9	65.3	0
4	*P. nigromaculatus*	466.3	205.6	46.8	0
5	*I. nebulosus*	212.8	113.6	38.5	0
6	*I. nebulosus*	387.5	106.3	15.6	0
8	*I. nebulosus*	290.9	190.7	42.4	0

[a]Mean concentration of diflubenzuron in water from all ponds 1 h after treatment.

The gastropods and oligochaetes are of interest because they contain some chitin (Rudall 1955). The density of gastropods in the treated ponds decreased by 86% during the first 8 d following treatment (Fig. 2). However, the natural interpond variation was so great that there were no significant differences ($P > 0.05$) between the control and the treated gastropod populations. Similarly, there were no significant differences between the control and the treated ponds in the density of oligochaetes (primarily Tubificidae) during the study.

The present study suggests that failure of an organism to reappear in a pond after being reduced by treatment does not necessarily indicate that diflubenzuron residues are preventing its reestablishment. For example, the *Daphnia* sp. population in pond 2 did not recover during the study although the *Daphnia* sp. in pond 6 became reestablished and exceeded pretreatment densities by 3 wk posttreatment. The absence of *Daphnia* sp. in pond 2 may be due to the lack of a chance reintroduction (e.g., by a bird), the lack of ephippial eggs, the abundance of a predator, or some other factor not associated with the diflubenzuron treatment.

Two types of fish were utilized to determine whether ecological differences between species would result in differences in the effects of diflubenzuron on the species. Young-of-the-year *P. nigromaculatus* are considered to be midwater zooplanktivores (Keast 1968) whereas *I. nebulosus* are largely benthic feeders (Moyle 1976).

Table 2 indicates that 1 d after treatment, *P. nigromaculatus* removed from the ponds had diflubenzuron residues as high as 466.3 ng/g, and *I. nebulosus* had maximum residues of 387.5 ng/g. Residues in both species of fish decreased to nondetectable concentrations by 7 d posttreatment.

The accumulation (residue concentration in the fish divided by residue concentration in the water) of diflubenzuron has exceeded 80× in lab studies of 5.0-

cm (total length) centrarchid fishes (Schaefer et al. 1979). In a previous field study of 7–12-cm (total length) centrarchids the accumulation was 71× (Apperson et al. 1978). The 4-cm (standard length) centrarchids in the present field study had an accumulation of 33×. The 3-cm (standard length) ictalurids of the present study had the relatively low diflubenzuron accumulation of 23×. In each study, the accumulation was temporary, and declined rapidly as the diflubenzuron residues in the water decreased.

Fish mortality inspections of the ponds indicated that means of 0.3 and 2.8 dead fish per pond per week were found during the periods 0–7 and 7–14 d pretreatment, respectively, in the treated (2, 4, 5, 6, and 8) ponds. In the control (1, 3, 7, and 9) ponds, 0.0 and 1.5 dead fish per pond per week were found during the periods 0–7 and 7–14 d before treatment, respectively. No dead fish were found in any ponds after the treatment date. These findings are in agreement with those of Takahashi and Miura (1975), who reported that a population of mosquitofish (*Gambusia affinis*) was able to survive five applications (each at 0.06 kg AI/ha) of diflubenzuron over a 5-mo period in field studies.

Although diflubenzuron does not directly cause fish mortality, the reductions in fish prey items might be important. The mean numbers of the predominant organisms present in the *P. nigromaculatus* stomach contents are plotted in Fig. 3. The mean number of cladocerans per stomach of fish in the treated ponds decreased by >99% within 7 d following treatment, and no noticeable increase occurred until more than 30 d posttreatment. Copepod counts in stomachs of the treated *P. nigromaculatus* decreased by 94% during the first 2 wk after the treatment, but had increased substantially by 3 wk posttreatment. The number of insects per fish from the treated ponds increased by 293% during the first 2 wk following the treatment. The insects that *P. nigromaculatus* were consuming after treatment were primarily baetid ephemeropterans (*Callibaetis* sp.), although the following also appeared in the fish stomachs: corixid hemipterans (*Hesperocorixa laevigata, Trichocorixa sp.*); notonectid hemipterans (*Notonecta undulata*); chironomid dipterans; corduliid odonatans (*Cordulia* sp.); coenagrionid odonatans (*Argia* sp.); and lestid odonatans (*Archilestes* sp.). The number of insects per stomach was also increasing in the control fish during this period, although these fish seemed to be feeding on smaller insect larvae. However, the difference in size (control mean length = 2.98 mm, treated mean length = 3.26 mm) measured on insects from stomach samples 7 d after treatment was not significant (*t*-test $P > 0.05$). The feeding habits of the treated fish eventually did return to normal, and there were no significant differences between control and treated *P. nigromaculatus* in the numbers of cladocerans, copepods, or insects consumed by 79 d posttreatment.

The stomach contents of *I. nebulosus* (Fig. 4) show

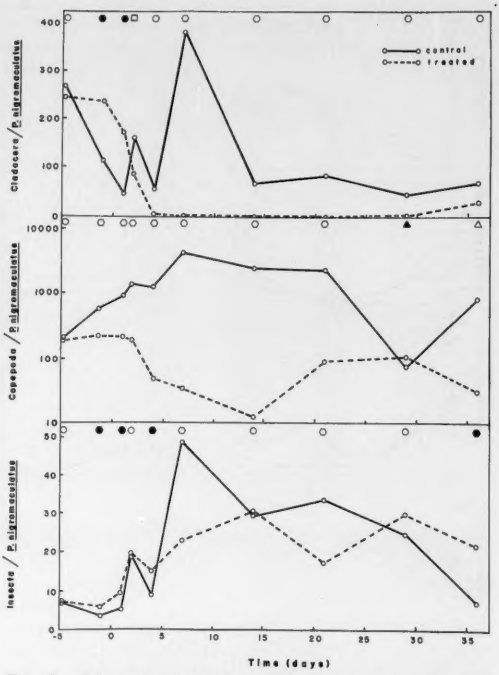

FIG. 3. Mean number of organisms per stomach of *P. nigromaculatus* from control (1, 3, 7, and 9) and treated (2, 4, 5, 6, and 8) ponds. Treatment occurred on day 0. Symbol indicates significance of difference between control and treated values; ○ = $P > 0.10$, □ = $0.10 < P > 0.05$, △ = $0.05 < P > 0.01$, ○ = $P < 0.01$. Solid symbol indicates treated value significantly greater than control value.

that cladocerans in the treated fish declined by 99% during the 1st wk posttreatment and did not begin to become important in the diet of the fish during the 1st mo posttreatment. The number of copepods per stomach decreased by 95% by 3 wk posttreatment and increased above pretreatment levels by 1 mo posttreatment. No major changes in the occurrence of insects in the *I. nebulosus* diet were observed. By 1 wk posttreatment, ostracods (primarily *Herpetocypris chevreuxi*) had become the most numerous organisms in the stomachs of the fish from the treated ponds. A laboratory study indicated ostracods can tolerate a diflubenzuron concentration of 500 μg/L (Miura and Takahashi 1975), but a field study indicated ostracods were depressed for 2 wk following treatment of 7 μg/L (Ali and Mulla 1978). During the present study, the density of ostracods remained sufficient to permit continued predation by *I. nebulosus*.

Some nematodes, gastropods (*Physa* sp., *Gyraulus* sp.), and oligochaetes (primarily Tubificidae) were also found in the stomachs of *I. nebulosus* removed from the ponds after the diflubenzuron treatment.

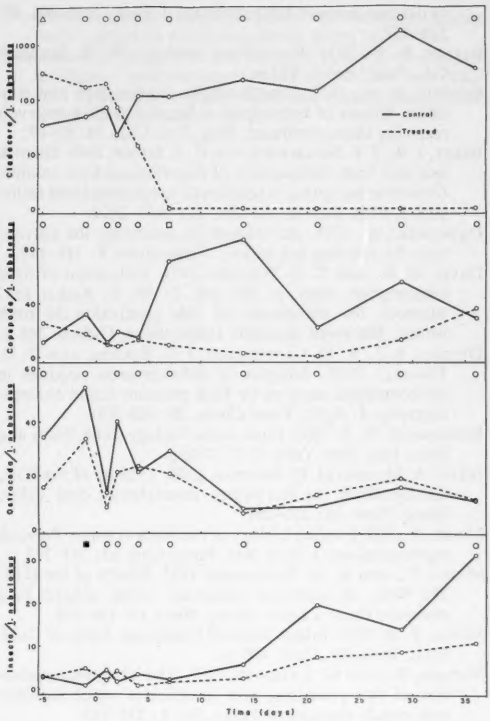

FIG. 4. Mean number of organisms per stomach of *I. nebulosus* from control (1, 3, 7, and 9) and treated (2, 4, 5, 6, and 8) ponds. Treatment occurred on day 0. Symbol indicates significance of difference between control and treated values; ○ = $P > 0.10$, □ = $0.10 < P > 0.05$, △ = $0.05 < P > 0.01$, ○ = $P < 0.01$. Solid symbol indicates treated value significantly greater than control value.

Regular sampling of the fish stomach contents was discontinued after 35 d but data taken on 79 d posttreatment indicated by this time that there were no significant differences between control and treated *I. nebulosus* in the numbers of cladocerans, copepods, insects, or ostracods consumed.

To determine whether or not the decreases in the numbers of some organisms were compensated by consumption of other organisms, stomach fullness indices were calculated according to the formula (Windell 1971):

$$\text{F. I. (‰)} = \frac{\text{fresh weight of stomach contents (g)} \times 10^4}{\text{fresh weight of fish (g)}}.$$

The mean fullness indices of treated pond *P. nigromaculatus* increased (from 299 to 379‰) during the first 2 d after treatment and then decreased and reached a low value (135‰) 2 wk posttreatment before recovery was initiated. During this same period control fullness indices ranged from 196 to 305‰. There were no significant (*t*-test at $P = 0.05$) differences between fish from control and treated ponds at any time during the study.

The percentage dry weight of the stomach contents is also important in assessing the biomass consumed by a fish (Davis and Warren 1971). The percentage dry weight of the stomach contents of *P. nigromaculatus* from the treated ponds increased 62% during the first 7 d posttreatment and remained above pretreatment levels through posttreatment day 35. During the same period, similar changes occurred for the control fish, and no significant (*t*-test at $P = 0.05$) difference occurred. On day 79, the percentage dry weight of stomach contents was 7% for the control and 8% for the treated *P. nigromaculatus* ($P > 0.05$).

For *I. nebulosus*, the stomach fullness index for the fish from the treated ponds reached its lowest value (161‰) 7 d after treatment, and this value was significantly (at $P = 0.05$) lower than the corresponding fullness index (244‰) for fish from the control ponds. Then the treated *I. nebulosus* fullness indices recovered to pretreatment levels and thereafter remained similar ($P > 0.05$) to those of the control fish. The percentage dry weight of stomach contents for *I. nebulosus* reached a low value (11%) on the day immediately following treatment, and exceeded pretreatment values by 1 wk after the treatment. No significant (at $P = 0.05$) differences in the percentage dry weight of stomach contents between control fish and treated fish occurred during the 79-d of the study.

The fish lengths and weights indicate how the growth rates varied after the diflubenzuron treatment. The control and treated *P. nigromaculatus* lengths were rather similar throughout the study. On 79 d posttreatment the mean standard length and mean weight of control fish were 66.4 mm and 7.4 g, respectively; the corresponding values for the treated *P. nigromaculatus* (74.6 mm and 10.9 g) were not significantly different (*t*-test $P > 0.05$).

Growth of *I. nebulosus* was also similar in the control and treated ponds. A *t*-test indicated that the difference between the lengths of the control fish (mean = 57.8 mm) and the treated fish (mean = 57.0 mm) on 79 d posttreatment was not significant ($P > 0.05$). Similarly, the weights of the control (mean = 4.3 g) and treated (mean = 4.2 g) *I. nebulosus* did not significantly differ ($P > 0.05$) at the termination of the study.

Fish receiving food rations which are deficient in quantity or quality may develop reduced percentage dry weights of their bodies within a few days (Brett et al. 1969; Dabrowski 1976). The percentage dry weight of *P. nigromaculatus* from the treated ponds actually increased during the 1st wk after the treatment and did not decline below pretreatment levels thereafter. On 79 d posttreatment, *P. nigromaculatus* from the control ponds had 22.1% dry weight, and those

from the treated ponds had 22.2% dry weight (t-test $P > 0.05$).

Percentage dry weights of *I. nebulosus* varied from 16.1 to 25.2% in the control ponds and from 17.1 to 19.4% in the treated ponds during the study. The t-tests indicated that the control *I. nebulosus* percentage dry weights were significantly (at $P = 0.05$) greater than the treated values on one sampling date (79 d posttreatment), and the treated values were significantly greater than the control values on two sampling dates (4 d and 7 d posttreatment).

The condition factor, an indicator of the fitness of a fish (Bennett 1972), was computed according to the following formula:

$$\text{C. F. } (K) = \frac{\text{dry weight of fish (g)} \times 10^7}{\text{standard length of fish (mm)}^3}.$$

Condition factors of *P. nigromaculatus* in the treated ponds increased (to 21) during the first 4 d after treatment and then decreased to a low level (17) about 3 wk posttreatment. On day 79 posttreatment, the condition factor was 18 for the control fish and 20 for the treated fish. The t-tests indicated during the study that there were no significant (at $P = 0.05$) differences between control and treated condition factors of *P. nigromaculatus*. Similarly, there were no significant (at $P = 0.05$) differences in condition factors of control and treated *I. nebulosus* after the diflubenzuron treatment.

The results of this study suggest that the peak levels of diflubenzuron in the upper water (Table 1) shortly after treatment were responsible for many of the observed effects. The fish (*P. nigromaculatus*) which were higher in the water column had a mean maximum diflubenzuron concentration 43% greater (Table 2) than that of the fish (*I. nebulosus*) residing lower in the water. Also, the aquatic invertebrates occurring relatively high in the treated water were significantly (at $P < 0.05$) reduced (Fig. 1) while none of the organisms occurring lower in the water or in benthos (Fig. 2) were significantly (at $P < 0.05$) reduced. However, even for *P. nigromaculatus*, the stomach fullness indices and condition factors of the treated fish were not significantly (at $P = 0.05$) reduced.

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Ali, A., and M. S. Mulla. 1978. Effects of chironomid larvicides and diflubenzuron on nontarget invertebrates in residential lakes. Environ. Entomol. 7: 21–27.

Apperson, C. S., C. H. Schaefer, A. E. Colwell, G. H. Werner, N. L. Anderson, E. F. Dupras Jr., and D. R. Longanecker. 1978. Effects of diflubenzuron on *Chaoborus astictopus* and nontarget organisms and persistence of diflubenzuron in lentic habitats. J. Econ. Entomol. 48: 521–527.

Barnes, R. D. 1974. Invertebrate zoology. W. B. Saunders Co., Philadelphia. 870 p.

Bennett, D. H. 1972. Length–weight relationships and condition factors of fishes from a South Carolina reservoir receiving thermal effluent. Prog. Fish Cult. 34: 85–87.

Brett, J. R., J. E. Shelbourn, and C. T. Shoop. 1969. Growth rate and body composition of fingerling sockeye salmon, *Oncorhynchus nerka*, in relation to temperature and ration size. J. Fish. Res. Board Can. 26: 2663–2694.

Dabrowski, K. 1976. An attempt to determine the survival time for starving fish larvae. Aquaculture 8: 189–193.

Davis, G. E., and C. E. Warren. 1971 Estimation of food consumption rates, p. 227–248. *In* W. E. Ricker [ed.] Methods for assessment of fish production in fresh waters. Blackwell Scientific Publications, Oxford. 348 p.

Diprima, S. J., R. D. Cannizzaro, J. C. Rogers, and C. D. Ferrell. 1978. Analysis of diflubenzuron residues in environmental samples by high pressure liquid chromatography. J. Agric. Food Chem. 26: 968–970.

Edmondson, W. T. 1963. Fresh-water biology. John Wiley and Sons, Inc., New York, N.Y. 1248 p.

Julin, A. M., and H. O. Sanders. 1978. Toxicity of the IGR, diflubenzuron, to freshwater invertebrates and fishes, Mosq. News 38: 256–259.

Keast, A. 1968. Feeding biology of the black crappie, *Pomoxis nigromaculatus*. J. Fish. Res. Board Can. 25: 285–297.

Miura, T., and R. M. Takahashi. 1975. Effects of the IGR, TH 6040, on nontarget organisms when utilized as a mosquito control agent. Mosq. News 35: 154–158.

Moyle, P B. 1976. Inland fishes of California. Univ. of Calif. Press, Berkeley, Calif. 405 p.

Mulder, R., and M. J. Gijwijt. 1973. The laboratory evaluation of two promising new insecticides which interfere with cuticle deposition. Pestic. Sci. 4: 737–745.

Mulla, M. S., G. Majori, and H. A. Darwazeh. 1975. Effects of the insect growth regulator Dimilin® or TH-6040 on mosquitoes and some nontarget organisms. Mosq. News 35: 211–216.

Post, L. C., and W. R. Vincent. 1973. A new insecticide inhibits chitin synthesis. Naturwissenschaften 60: 431–432.

Rodriguez, C. S., and R. E. Wright. 1978. Evaluation of the insect growth regulators methoprene and diflubenzuron against floodwater mosquitoes (Diptera: Culicidae) in southwestern Ontario. Can. Entomol. 100: 319–324.

Rudall, K. M. 1955. The distribution of collagen and chitin. Symp. Soc. Exp. Biol. 9: 49–71.

Schaefer, C. H., and E. F. Dupras Jr. 1977. Residues of diflubenzuron 1-(4-chlorophenyl)-3-(2, 6 difluorobenzoyl)-urea in pasture soil, vegetation and water following aerial applications. J. Agric. Food Chem. 25: 1026–1030.

Schaefer, C. H., E. F. Dupras Jr., R. J. Stewart, L. W. Davidson, and A. E. Colwell. 1979. The accumulation and elimination of diflubenzuron by fish. Bull. Environ. Contam. Toxicol. 21: 249–254.

Schaefer, C. H., and W. H. Wilder. 1973. Insect developmental inhibitors. 2. Effects on target mosquito species. J. Econ. Entomol. 6: 913–916.

Snell, J. B., and W. Hazeltine. 1963. The use of insecticide to determine the life history of an aquatic gnat *Chaoborus astictopus*. Ann. Entomol. Soc. Am. 56: 816–818.

Steelman, C. D., J. E. Farlow, T. P. Breaud, and P. E. Schilling. 1975. Effects of growth regulators on *Psoro-*

phora columbiae (Dyar and Knab) and nontarget aquatic insect species in rice fields. Mosq. News 35: 67–76.

TAKAHASHI, R. M., AND T. MIURA. 1975. Insect developmental inhibitors: multiple applications of Dimilin® and Altosid® to *Gambusia affinis* (Baird and Girard) Proc. Calif. Mosq. Control Assoc. 43: 85–87.

USINGER, R. L. [ed.] 1956. Aquatic insects of California. Univ. of Calif. Press, Berkeley, Calif. 508 p.

WINDELL, J. T. 1971. Food analysis and rate of digestion, p. 215–225. *In* W. E. Ricker [ed.] Methods for assessment of fish production in fresh waters. Blackwell Scientific Publications, Oxford. 348 p.

Direct Determination of Apatite in Lake Sediments

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Apatite is a common accessory mineral in the source rocks for the glacial debris supplying sediments to many Canadian lakes. A method has been developed which uses scanning electron microscopy and energy dispersive X-ray emission spectrometry for direct identification of apatite. This method has been used to examine the apatite content of various size fractions in Kamloops Lake sediments. Apatite concentrations obtained by this direct examination correlate well ($r > 0.999$) with apatite concentrations determined by chemical analyses and indicate that, in addition to comprising as much as 70% of the total phosphorus load, apatite may comprise as much as one-third of the "dissolved" (<0.45 μm) inorganic phosphorus load. Consequently the use of classical (e.g. Vollenweider 1968; Vollenweider and Dillon 1974) methods of estimating lake trophic state from inorganic phosphorus concentrations in lake water must be done with care, recognizing that the bulk of inorganic phosphorus in lakes deriving sediment from glaciated igneous or metamorphic terrains may be in the form of apatite.

Key words: apatite, lake, trophic state, phosphorus load, scanning electron microscopy

REID, R. P., C. H. PHARO, AND W. C. BARNES. 1980. Direct determination of apatite in lake sediments. Can. J. Fish. Aquat. Sci. 37: 640–646.

L'apatite est un minéral accessoire communément présent dans les roches d'où naissent les débris glaciaires fournissant les sédiments à plusieurs lacs canadiens. Les auteurs ont mis au point une méthode faisant appel à la microscopie électronique à balayage et à la spectrométrie à émission de rayons-X dispersifs d'énergie pour l'identification directe de l'apatite. On s'est servi de cette méthode pour déterminer la teneur en apatite de fractions de tailles diverses dans les sédiments du lac Kamloops. Il y a bonne corrélation ($r > 0{,}999$) entre les concentrations d'apatite trouvées par cet examen direct et les concentrations d'apatite déterminées par analyses chimiques. Ceci veut dire qu'en plus d'inclure jusqu'à 75% de la charge de phosphore total, l'apatite peut comprendre jusqu'à un tiers de la charge de phosphore inorganique «dissous» (<0,45 μm). C'est pourquoi il faut utiliser avec prudence les méthodes classiques (e.g. Vollenweider 1968; Vollenweider et Dillon 1974) d'estimation de l'état trophique d'un lac à partir des concentrations de phosphore inorganique dans l'eau, réalisant que la plus grande partie du phosphore inorganique présent dans les lacs recevant leur sédiment de terrains métamorphiques ou ignés ayant subi l'action glaciaire peut être sous forme d'apatite.

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RECENT investigations of forms of phosphorus in lake sediments have shown that a large portion of the total phosphorus in some lakes is present as apatite (Williams and Mayer 1972; St. John et al. 1976). The most abundant form of primary apatite in nature is fluorapatite [$Ca_5(PO_4)_3F$] (Williams et al. 1976; Deer et al. 1966) and because the solubility of fluorapatite in lake water is extremely low, it is unlikely to be more than a minor source of phosphorus for biological growth (Syers et al. 1973; Sagher et al. 1975; Williams et al. 1976). Sagher et al. (1975) investigated the availability of sediment phosphorus and concluded that non-occluded Fe-bound phosphorus was the most available form of sediment phosphorus, whereas apatite phosphorus was of limited availability. Syers et al. (1973) conclude that Ca-P (apatite) in lake water is essentially immobile, and Williams et al. (1976) commented that a high input of apatite-P to Lake Erie prior to 1850 did not render this lake eutrophic. However, Smith et al. (1978) have shown that naturally occurring apatites may provide a source of orthophosphate for growth of

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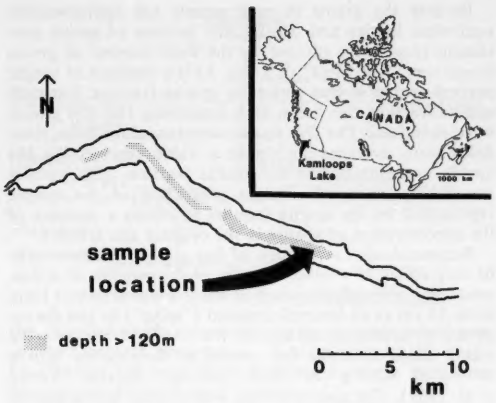

FIG. 1. Sediment sample site, Kamloops Lake.

bacteria and algae. At present, the question of how much phosphorus detrital apatite contributes to the nutrient budget of a lake remains unresolved. If apatite phosphorus is largely unavailable for biological growth, it should not be included in the total phosphorus loading values used to estimate trophic state. This problem was recognized by Vollenweider (1968), Dillon and Kirchner (1975), and Peters (1978).

Kamloops Lake, the subject of the present study, is a 2.1 km × 25 km fjord lake which occupies a glacial valley in the semiarid, south-central region of British Columbia (Fig. 1). Chemical analyses of water and sediment in the lake show that 70% of the total phosphorus entering the lake is in the form of apatite and that apatite may occur in all size fractions of the lake sediment (St. John et al. 1976). This is significant, for if apatite is present in the lake as particles smaller than 0.45 μm, it will pass through the membrane filters used to separate "dissolved" from "particulate" materials and be included in the "dissolved" phosphorus pool. Therefore, estimates of productivity in this lake could be erroneous even if dissolved rather than total phosphorus values are used for the estimation. Both Williams et al. (1976) and Smith et al. (1978) state that the solubility of apatite increases with decreasing particle size, but no estimates have been made of the availability of phosphorus in apatite that is smaller than 0.45 μm.

The method used to identify apatite in the 1976 Kamloops Lake study (St. John et al. 1976) was the selective chemical extraction technique of Williams et al. (1976). There are, however, problems associated with specificity of the colorimetric method for analysis of orthophosphate (Harwood and Hattingh 1973). Complexes of silicate, arsenate, and germanate can form heteropoly acids with molybdenum and interfere with the phosphorus determination. Labile organic phosphorus compounds will also interfere, as they are hydrolyzed in the extraction procedure (Downes and Paerl 1978; Peters 1978). In general, the chemical extraction technique for identifying forms of phosphorus in soils and sediments has developed as a result of extracting known compounds in the laboratory (Fisher and Thomas 1935; Williams 1937; Dean 1938; Williams 1950; Chang and Jackson 1957; Williams et al. 1967, 1971a, 1971b).

Direct methods for identifying and quantifying phosphate minerals in soils and sediments have met with limited success because of the small quantities of these minerals and because of difficulties in petrographic determination (Shipp and Matelski 1960; Sawhney 1973; Lindsay and Vlek 1977). Kingston (P. W. Kingston, Environment Canada, Canada Centre for Inland Waters, 1973, unpublished data) examined the "feasibility of detecting and quantitatively estimating phosphate minerals in lacustrine sediments by mineralogical methods." He concentrated phosphate grains by heavy liquid and magnetic separations and suggested that the microprobe could be used effectively to obtain particle size distribution, relative abundance, and mineralogy of discrete phosphate grains. However, Kingston's method is not quantitative.

The purpose of the present study was to develop a method of physical separation, identification, and quantification of apatite in lake sediments, to use this method to investigate the size distribution of apatite in Kamloops Lake sediments and to determine the efficiency of selective chemical extraction as a method of quantifying the apatite phosphorus content of lake sediments. In addition, an attempt was made to estimate the amount of apatite which contributes to the "dissolved" phosphorus load of Kamloops Lake.

Methods

Physical Separation, Identification, and Quantification of Apatite

A sample of sediment was collected with a Shipek grab sampler at a depth of 130 m at the east end of the lake (Fig. 1) in the pro-delta region of the Thompson River Delta. The particle size distribution of the total sediment was determined by hydrometer analysis. The sample was divided into eight size fractions from 125 to <2 μm: a 125–62-μm fraction was obtained by sieving; five fractions between 62 and 10 μm were separated in a hydraulic cyclone elutriator; and a 10–2-μm and a <2-μm fraction were obtained by centrifugation. Size analyses of the six fractions from 62 to 2 μm were performed using a Sedigraph 5000 fine-particle size analyzer by the National Water Research Institute (NWRI), West Vancouver, to check the efficiency of the size separation procedure.

Each of the eight size fractions was further subdivided by density centrifugation; 250 mg of sediment was centrifuged in 50 mL of diiodomethane to remove the particles with density >3.3 $g \cdot cm^{-3}$ (Fig. 2). The light particles from this separation were then centrifuged in 50 mL of acetone–diiodomethane solution with a density of 3.05 $g \cdot cm^{-3}$. Apatite was concentrated in the heavy fraction, henceforth called the 'apatite fraction,' which contains minerals with

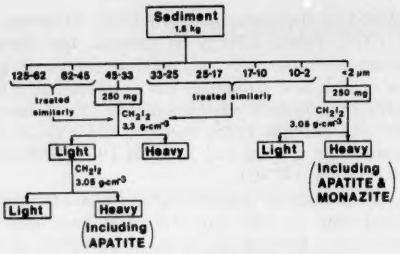

FIG. 2. Heavy liquid separation scheme.

densities ranging between 3.05 $g \cdot cm^{-3}$ and 3.3 $g \cdot cm^{-3}$. The theoretical density of fluorapatite is 3.20 $g \cdot cm^{-3}$ (McConnell 1973); however, isomorphous substitutions in naturally occurring fluorapatites result in a range of densities from 3.1 $g \cdot cm^{-3}$ to 3.2 $g \cdot cm^{-3}$ (Mason and Berry 1968). Densities of some igneous apatites reported by McConnell (1973) range from 3.17 $g \cdot cm^{-3}$ to 3.22 $g \cdot cm^{-3}$.

The separation procedure described above was followed for all size fractions of the Kamloops Lake sediment except the <2-μm fraction. Because of the very long centrifugation times required for separation of the <2-μm fraction, only the 3.05 $g \cdot cm^{-3}$ separation was done. A detailed description of the separation procedure is given in Reid (1979). Triplicate or quadruplicate heavy liquid separations of the five size fractions from 62 to 10 μm were performed; single separations were done for the other three size fractions.

A subsample of each apatite fraction obtained by the heavy liquid separation was mounted on a graphite disc and examined at low magnification with an ETEC Autoscan scanning electron microscope (SEM) equipped with an ORTEC X-ray analytical system. Grains in the apatite fractions that contain phosphorus are readily identified in the X-ray image of the phosphorus Kα emission (Fig. 3). Once identified, the phosphorus-containing grains were examined individually and their elemental compositions determined from their X-ray emission spectra.

Because the grains in each sample are approximately equivalent in size and density, the number of grains containing phosphorus divided by the total number of grains in any one SEM "field" (e.g. Fig. 3) is a measure of weight percent apatite in that particular apatite fraction. For each apatite fraction, five fields, each containing 100–200 grains, were examined. The five apatite concentrations from these fields were averaged to obtain a value representing the apatite concentration in the apatite fraction. This number was multiplied by the weight proportion of the sample represented by the apatite fraction to obtain a measure of the concentration of apatite in the original size fraction.

Because density separation of fine-grained sediment may be incomplete, a weighed amount of fluorapatite of appropriate size was added to each of the five size fractions from 62 to 10 μm as an internal standard ("spike") to test the recovery of apatite in the apatite fraction. The apatite spike was a single inclusion-free crystal of fluorapatite with a density of 3.216 $g \cdot cm^{-3}$ from Durango, Mexico (Young et al. 1969). The apatite crystal was crushed and separated into the five size fractions by gravity settling and decantation. As in the unspiked samples for these five size fractions, triplicate or quadruplicate analyses (both heavy liquid separation and S.E.M. examination) were performed. Spiked and unspiked samples of the 62–45-μm sediment from Kamloops Lake were separated with heavy liquids, as above, after being extracted for non-apatite inorganic phosphorus (NAIP) and apatite, and were examined with the scanning electron microscope.

Selective Chemical Extraction of Apatite

Selective chemical extractions of apatite for this study were performed by NWRI, West Vancouver. The extraction scheme of Williams et al. (1976) was modified to use 1 M HCl, rather than 0.5 M HCl, for the apatite extraction (see Fig. 4). Initial verification of the technique using apatite standards was followed by extraction of seven of the size fractions of Kamloops Lake sediments. Verification of the selective chemical extraction technique involved extraction of an apatite standard, extraction of sediment con-

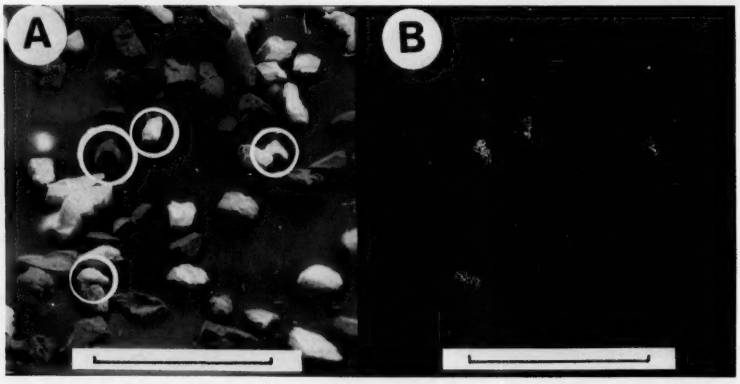

FIG. 3. Identification of phosphorus-containing grains with a scanning electron microscope equipped with an energy dispersive X-ray emission spectrometer. (A) SEM image; apatite grains are circled. (B) Phosphorus Kα image. Bars are 500 μm long.

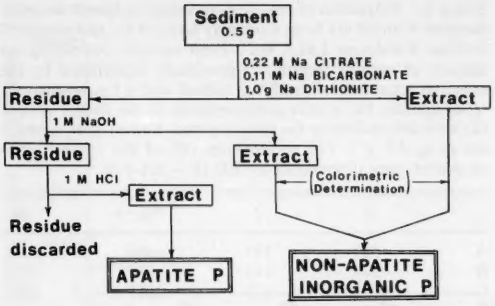

FIG. 4. Extraction scheme for apatite phosphorus and non-apatite inorganic phosphorus (NAIP) used in this study. Modified by National Water Research Institute, West Vancouver, from Williams et al. (1976).

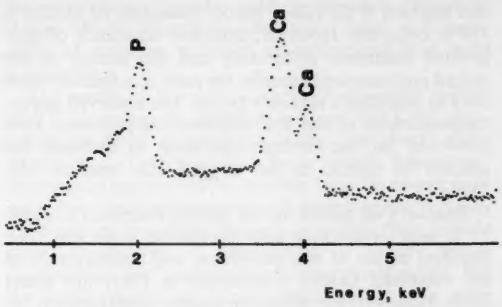

FIG. 5. X-ray emission spectrum for Kamloops Lake apatite.

taining apatite as an internal standard, and S.E.M. examination of the extracted sediment. The apatite standard was the same crushed fluorapatite crystal used in the physical separation spiking procedure.

Results and Discussion

PHYSICAL SEPARATION

Phosphorus-containing grains observed in all size fractions, with the exception of the >2-μm fraction, contained Ca and P in proportions equivalent to those observed in an apatite standard. Some grains had X-ray emission spectra indicating the additional presence of minor amounts of Al, Si, and/or Fe, that are probably present as coatings on the apatite, as the grains were not treated to remove sesquioxides. The numbers of grains with these coatings increased slightly in the <10-μm sizes. A typical X-ray emission spectrum for the phosphorus-containing grains is shown in Fig. 5. The X-ray energy from fluorine is not detected by the emission method used.

Mean observed apatite concentrations ($\bar{M}_{ob}$ and $\bar{K}_{ob}$) for each of the five fractions from 62 to 10 μm are summarized in Table 1. The standard error of each mean observed apatite concentration is ~5%, and reflects differences between subsamples and variability in estimates of the percent apatite in the apatite fraction.

The mean observed apatite concentration, $\bar{M}_{ob}$, of the unspiked sample represents the amount of apatite in the

TABLE 1. Mean observed apatite concentrations determined by physical separation. ($\bar{M}_{ob}$: unspiked sample; $\bar{K}_{ob}$: spiked sample; I: internal standard; p: recovery factor; M: apatite concentrations rounded to two significant figures). All values except "p" are expressed as $\mu g \cdot g^{-1}$.

Particle size, μm	$\bar{M}_{ob}$	$\bar{K}_{ob}$	I	p	M
62–45	7080 ± 320	11 430 ± 590	4616	0.94 ± 0.15	7500 ± 1400
45–33	4600 ± 180	7 280 ± 530	2795	0.96 ± 0.20	4800 ± 1100
33–25	3430 ± 130	6 220 ± 210	3401	0.82 ± 0.07	4200 ± 500
25–17	3260 ± 240	5 350 ± 610	2699	0.77 ± 0.24	4200 ± 1500
17–10	2220 ± 200	3 790 ± 150	2264	0.69 ± 0.11	3200 ± 500
10–2	1030 (single observation)				

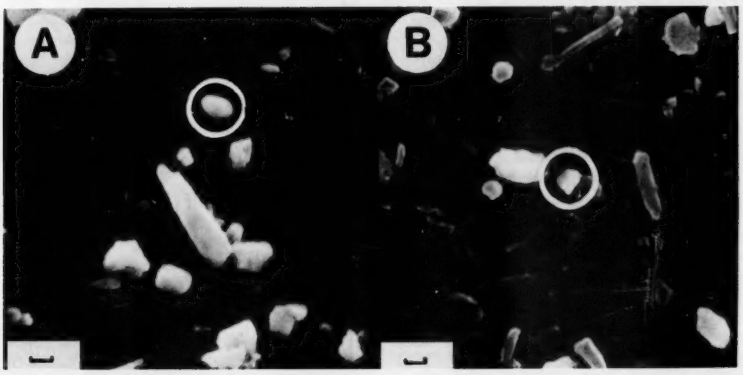

FIG. 6. Apatite (A) and monazite (B) grains with long diameters near 0.45 μm are circled. Bars are 0.45 μm long.

size fraction if the heavy liquid separation of apatite is 100% complete. However, complete separation of fine-grained sediments is unlikely and the means of the spiked and unspiked samples for each size fraction were used to calculate a recovery factor. The observed apatite concentrations of the five unspiked samples were then corrected for incomplete separation to estimate the amount of apatite in the original size fraction (M, Table 1).

Recovery of apatite in the apatite fraction (p, Table 1) is seen to decrease with decreasing grain size. The standard errors of the recoveries, and consequently of the corrected apatite concentrations (M), are about 20%. However, the minimum apatite concentration for any size fraction is that observed in the unspiked fraction ($\bar{M}_{ob}$), with its smaller error.

No attempt was made to quantify the apatite in the <2-μm fraction by point-counting phosphorus-containing grains due to difficulties in detection of small grains by X-ray spectrometry. Because only a 3.05-g·cm^{-3} density separation was attempted for this fraction, the apatite in the <2-μm fraction was less concentrated than in the apatite fractions for the other grain sizes. On the other hand, phosphorus-containing grains such as monazite, a cerium–lanthanum phosphate, which would not be included in the apatite fraction of other size ranges, were detected in this sample (Fig. 6). Like apatite, it is very insoluble and therefore is unlikely to be biologically utilized to a significant extent.

Chemical Extraction

The results of the chemical extraction of apatite standards and spiked sediment samples are presented in Tables 2 and 3. The phosphorus content of the apatite was assumed to be 17.8% (Young et al. 1969). Errors involved in weighing the apatite are less than 0.5%.

Recoveries of the apatite standard ranged from 86 to 97%, with 2–5% of the apatite being extracted as NAIP (Table 2). Recoveries of apatite in the spiked sediment ranged from 75 to 103% (Table 3). These values are well within the range of those expected, as the standard error of the mean of the apatite measured by the selective chemical extraction method in both spiked and unspiked samples is ~3%.

No apatite grains were observed in the apatite fraction, suggesting that all of the apatite was indeed dissolved in the extraction. In general, the Williams et al. (1976) technique of selective chemical extraction of apatite seems to be effective in recovering a fluorapatite standard. Wildung et al. (1977) found that the Williams method generally gave recoveries of >90% of inorganic phosphorus added to lake sediments.

The apatite concentrations obtained by chemical extraction are plotted against those of the corrected physical separation in Fig. 7. The two methods give similar results for the five size fractions from 62 to 10 μm. The 10–2-μm point-count value is considerably lower than the extraction value because this sample was not spiked to estimate recovery.

Because the point-count value for the 10–2-μm sedi-

Table 2. Results of the selective chemical extraction of an apatite standard using a minor modification of the method of Williams et al. (1976). A single crystal of apatite was crushed and part was separated into three size fractions, with initial weights (W) expressed as μg P. Nonapatite inorganic phosphorus (NAIP in μg P) and apatite phosphorus (AP in μg P) are also expressed as percentages of the initial weight of apatite phosphorus extracted.

Size, μm	W	NAIP	NAIP (%)	AP	AP (%)
All	2081	105	5%	1800	86%
10–2	2183	85	4%	2100	96%
25–17	2096	N/E[a]		2000	95%
33–25	2580	64	2%	2500	97%

[a]Not extracted.

Table 3. Extraction of apatite from spiked sediment samples. Samples A and B are from Kootenay Lake, B.C., and sample C is from Kamloops Lake, B.C. Each sample, containing an amount of natural apatite (U) previously determined by the selective extraction method, was spiked with a known amount (I) of apatite; the apatite concentrations in the spiked sample (S) were determined by the same method. Values of U, I, and S are in μg AP·g^{-1}. The proportions (R) of the apatite spike recovered were determined by 100 $(S - U)\cdot I^{-1}$.

	U	I	S	R
A	549	143	696	103%
B	549	148	660	75%
C	1293	821	2017	88%

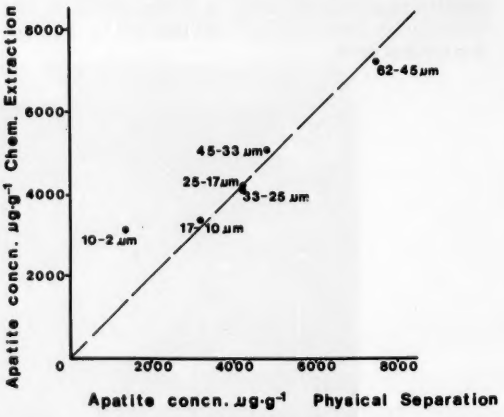

Fig. 7. Correlation between apatite concentration obtained by chemical extraction and that obtained by physical separation (after correction for incomplete recovery). Broken line equals perfect correlation.

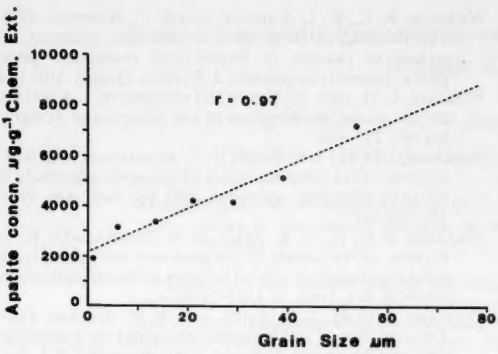

FIG. 8. Relation between apatite concentration and grain size, plotted as the means of seven sediment size fractions, for Kamloops Lake sediments.

ment has not been corrected for incomplete separation, the chemical extraction rather than the physical separation data are plotted in Fig. 8 to estimate the concentration of apatite in sediment finer than 0.45 μm. Extrapolation of the extraction data suggests that the concentration at 0.45 μm may be as high as 2200 μg apatite g^{-1}. Therefore, apatite smaller than 0.45 μm probably contributes to the "dissolved" phosphorus load in Kamloops Lake. The proportion of the "dissolved" phosphorus load contributed by microparticulate (<0.45 μm) apatite cannot be calculated with certainty from the data now available, but an approximate minimum value may be estimated. The total sediment input to the lake, estimated from varve thicknesses, is 1.4 $Tg \cdot a^{-1}$. As grain size analyses of surface samples suggest that at least 7% of the sediment input to the lake is <0.45 μm in diameter, the microparticulate inorganic input is 98 $Gg \cdot a^{-1}$. If the concentration of microparticulate apatite in the <45-μm sediment fraction is 2200 $\mu g \cdot g^{-1}$, then the input of microparticulate apatite to the lake is 220 $Mg \cdot a^{-1}$. As fluorapatite is 18% phosphorus, this represents an input of 39 Mg $P \cdot a^{-1}$, which is approximately one-third of the 118 $Mg \cdot a^{-1}$ "dissolved" P input to the lake reported by St. John et al. (1976).

In conclusion it can be stated that a method has been developed which shows that the concentrations of apatite obtained by indirect chemical extraction of phosphorus in Kamloops Lake sediments are comparable to the values obtained by direct physical observation. The method is time consuming, tedious, and expensive, and is not recommended as a general procedure for evaluating concentrations of apatite in sediments. Rather, it was used to verify "apatite" concentrations from chemical extractions, and could, of course, be used for identification and quantification of other heavy minerals. The scanning electron microscope equipped with energy dispersive X-ray spectrometer has proven to be extremely useful, and may be more versatile than the electron microprobe used in previous phosphate studies (P. W. Kingston unpublished data) as it is capable of scanning at lower magnifications than most microprobes.

The results of this study indicate that apatite smaller than 0.45 μm may be abundant in Kamloops Lake, comprising as much as one-third of the "dissolved" phosphorus load. Total and, to a lesser extent, "dissolved" phosphorus measurements in other lakes receiving sediment from glaciated igneous and metamorphic terranes are likely to be of little value in assessing their trophic state. Availability of phosphorus in apatite grains smaller than 0.45 μm requires further investigation, but the linear relation of apatite concentration with grain size (Fig. 8) suggests that apatite is not being significantly utilized by primary producers in Kamloops Lake.

Acknowledgments

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CHANG, S. C., AND M. L. JACKSON. 1957. Fractionation of soil phosphorus. Soil Sci. 84: 133–144.

DEAN, L. A. 1938. An attempted fraction of the soil phosphorus. J. Agric. Sci. 28: 234–246.

DEER, W. A., R. A. HOWIE, AND J. ZUSSMAN. 1966. An introduction to the rock forming minerals. Longmans, Green and Co., Ltd., London. p. 504–509.

DILLON, P. J., AND W. B. KIRCHNER. 1975. The effects of geology and land use on the export of phosphorus from watersheds. Water Res. 9: 135–148.

DOWNES, M. T., AND H. W. PAERL. 1978. Separation of two dissolved reactive phosphorus fractions in lakewater. J. Fish. Res. Board Can. 35: 1636–1639.

FISHER, R. A., AND R. P. THOMAS. 1935. The determination of the forms of inorganic phosphorus in soils. J. Am. Soc. Agron. 27: 863–873.

HARWOOD, J. E., AND W. H. J. HATTINGH. 1973. Colorimetric methods of analysis of phosphorus at low concentrations in water, p. 289–301. *In* E. J. Griffith, A. Beeton, J. M. Spencer, and D. T. M. Mitchell [ed.] The environmental phosphorus handbook. John Wiley and Sons, Inc., New York, N.Y.

LINDSAY, W. L., AND P. L. G. VLEK. 1977. Phosphate minerals, p. 639–672. *In* Minerals in soil environments. Soil Sci. Soc. Am.

MASON, B., AND L. G. BERRY. 1968. Elements of mineralogy. W. H. Freeman, San Francisco, Calif. 550 p.

MCCONNELL, D. 1973. Apatite. Springer-Verlag, New York, N.Y. 111 p.

PETERS, R. H. 1978. Concentrations and kinetics of phosphorus fractions in water from streams entering Lake Memphremagog. J. Fish. Res. Board Can. 35: 315–328.

REID, R. P. 1979. Apatite in a glacial lake. M.Sc. thesis, Univ. British Columbia, Vancouver, B.C. 42 p.

SAGHER, A., R. F. HARRIS, AND D. E. ARMSTRONG. 1975. Availability of sediment phosphorus to microorganisms. Water Resources Center, Univ. Wisc. Tech. Rep. WIS-WRC 75-01: 57 p.

SAWHNEY, B. L. 1973. Electron microprobe analysis of phosphates in soils and sediments. Soil Sci. Soc. Am. Proc. 37: 658–660.

SHIPP, R. F., AND R. P. MATELSKI. 1960. A microscopic determination of apatite and a study of phosphorus in some Nebraska soil profiles. Soil Sci. Soc. Am. Proc. 24: 450–452.

SMITH, E. A., C. I. MAYFIELD, AND P. T. S. WONG. 1978. Naturally occurring apatite as a source of orthophosphate for growth of bacteria and algae. Microb. Ecol. 4: 105–117.

ST. JOHN, B. E., E. C. CARMACK, R. J. DALEY, C. B. J. GRAY, AND C. H. PHARO. 1976. The limnology of Kamloops Lake, British Columbia. Environment Canada, Inland Waters Directorate, Pacific and Yukon Region, Vancouver, B.C. 167 p.

SYERS, J. K., R. F. HARRIS, AND D. E. ARMSTRONG. 1973. Phosphate chemistry in lake sediments. J. Environ. Qual. 2: 1–14.

VOLLENWEIDER, R. A. 1968. Scientific fundamentals of the eutrophication of lakes and flowing waters, with particular reference to nitrogen and phosphorus as factors in eutrophication. Tech. Rep. United Nations Organization for Economic Cooperation and Development, Paris. 192 p.

VOLLENWEIDER, R. A., AND P. J. DILLON. 1974. The application of the phosphorus loading concept to eutrophication research. National Research Council of Canada, Associated Committee on Scientific Criteria for Environmental Quality, Rep. NRCC 13690: 42 p.

WILDUNG, R. E., R. L. SCHMIDT, AND R. C. ROUTSON. 1977. The phosphorus status of eutrophic lake sediments as related to changes in limnological conditions—phosphorus mineral components. J. Environ. Qual. 6: 100–104.

WILLIAMS, C. H. 1950. Studies on soil phosphorus. I. A method for the partial fractionation of soil phosphorus. J. Agric. Sci. 40: 233–242.

WILLIAMS, J. D. H., J. K. SYERS, D. E. ARMSTRONG, AND R. F. HARRIS. 1971a. Characteristics of inorganic phosphate in noncalcareous lake sediments. Soil Sci. Soc. Am. Proc. 35: 556–561.

WILLIAMS, J. D. H., J. K. SYERS, S. S. SHUKLA, AND R. F. HARRIS. 1971b. Levels of inorganic and total phosphorus in lake sediments as related to other sediment parameters. Environ. Sci. Tech. 5: 1113–1120.

WILLIAMS, J. D. H., J. K. SYERS, AND T. W. WALKER. 1967. Fractionation of soil inorganic phosphate by a modification of Chang and Jackson's procedure. Soil Sci. Soc. Am. Proc. 31: 736–739.

WILLIAMS, J. D. H., J. M. JAQUET, AND R. L. THOMAS. 1976. Forms of phosphorus in the surficial sediments of Lake Erie. J. Fish. Res. Board Can. 33: 413–429.

WILLIAMS, J. D. H., AND T. MAYER. 1972. Effects of sediment diagenesis and regeneration of phosphorus with special reference to Lakes Erie and Ontario, p. 281–315. *In* H. E. Allen and J. R. Kramer [ed.] Nutrients in natural waters. Wiley-Interscience, New York, N.Y.

WILLIAMS, R. W. 1937. The solubility of soil phosphorus and other phosphorus compounds in sodium hydroxide solutions. J. Agric. Sci. 27: 259–270.

YOUNG, E. J., A. T. MYERS, E. L. MUNSON, AND N. M. CONKLIN. 1969. Mineralogy and geochemistry of fluorapatite from Cerro de Mercado, Durango, Mexico. United States Geological Survey Professional Paper 650-D. p. 84–93.

Insect Community Structure as an Index of Heavy-Metal Pollution in Lotic Ecosystems

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Data from two streams suggest that macroinvertebrate community structure exhibits a predictable, graded response to heavy-metal pollution. In the more heavily stressed sections of both streams, macroinvertebrates, other than tubificid worms and chironomids, were virtually eliminated from rock-rubble, riffle habitats. Midge larvae comprised 75 to 86% of all insects collected from the most grossly polluted stations and less than 10% of the insect communities at the least polluted stations. The correlation coefficient for percentage of chironomids in relation to copper concentration was +0.93 ($P \leq 0.01$). Changes in the coefficient of variation (CV) of percentage of chironomids per sample exhibited a consistent pattern. The lowest CV was at the most polluted stations and the CV increased along a gradient of decreasing metal concentrations, reaching maximum values at the least polluted stations. The number of species of chironomids was inversely related to the proportional density of the family in samples. In Elam's Run, the number of species of chironomids identified in samples increased from 15 at the most polluted station to 39 at the least polluted stations. Fifty-seven species of midges were collected along the 3-km stretch of this grossly polluted stream. Caddisflies were numerically important at the intermediately polluted stations in both streams. Mayflies occurred only at the least polluted stations. We hypothesize that heavily polluted habitats are dominated by midges, moderately polluted habitats by midges and caddisflies, and minimally polluted or unpolluted habitats by caddisflies and mayflies. Since there appears to be a direct relationship between the fraction of a community composed of chironomids and the degree of pollution, we suggest that the percentage of chironomids in samples may be a useful index of heavy-metal pollution.

Key words: heavy metals, stream pollution, chironomids, aquatic insects

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Les données recueillies dans deux cours d'eau laissent penser que la structure des communautés de macro-invertébrés réagit de manière graduée et prévisible à la pollution par métaux lourds. Dans les secteurs les plus pollués des deux cours d'eau, les macro-invertébrés autres que les vers tubificides et les chironomides sont virtuellement éliminés des habitats de roche et de pierraille des radiers. Les larves de moucherons constituent 75 à 86% de tous les insectes recueillis aux stations les plus fortement polluées, et moins de 10% des communautés d'insectes aux stations les moins polluées. Le coefficient de corrélation du % de chironomides par rapport à la concentration de cuivre est +0,93 ($P \leq 0{,}01$). Le coefficient de variation (CV) du % de chironomides par échantillon change selon un schéma uniforme. Le CV le plus bas se trouve aux stations les plus polluées et il augmente le long d'un gradient de concentration décroissante de métaux, atteignant des valeurs maximales aux stations les moins polluées. Le nombre des espèces de chironomides est en relation inverse de la densité proportionnelle de la famille dans les échantillons. Dans l'Elam's Run, le nombre des espèces de chironomides identifiées dans les échantillons augmente, passant de 15 à la station la plus polluée à 39 aux stations les moins polluées. Nous avons recueilli 57 espèces de moucherons le long d'un secteur de 3 km de ce cours d'eau fortement pollué. En nombre, les phryganes sont importantes aux stations de pollution intermédiaire dans les deux cours d'eau. Les éphémères ne sont présentes qu'aux stations les moins polluées. Nous mettons de l'avant l'hypothèse que les habitats fortement pollués sont dominés par des moucherons, les habitats modérément pollués, par des moucherons et des phryganes et enfin les habitats à pollution minimale ou nulle, par des

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phryganes et des éphémères. Comme il semble y avoir une relation directe entre la fraction de la communauté composée de chironomides et le degré de pollution, le % de chironomides dans les échantillons pourrait être un indice utile de pollution par les métaux lourds.

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WATER quality criteria, which specify allowable concentrations of heavy metals as a mechanism for protecting freshwater biota, are largely based on laboratory-derived toxicity tests with a few species of fish and an even smaller number of species of lentic invertebrates (U.S. Environmental Protection Agency 1976; Clarke 1974). Since most lotic invertebrates are notoriously difficult to maintain in the laboratory, water-quality criteria for lotic ecosystems should be related to field data on their response to particular chemicals. There are relatively few studies which document the response of lotic communities to heavy-metal stress (Södergren 1976; Sprague et al. 1965; Winner et al. 1975) and none of these has led to any generally acceptable criteria for evaluating the degree to which such systems have been biologically degraded.

In this paper, we present data on the response of macroinvertebrate communities to heavy-metal pollution in two streams. These data are related to published information on the composition of macroinvertebrate community structure in unpolluted and heavy-metal-polluted streams to derive a generalization about the response of such communities to varying levels of heavy-metal stress.

Study Areas

Data were collected from two 2nd order, limestone streams in southwestern Ohio. Shayler Run was used by the Newtown Fish Toxicology Station of the U.S. Environmental Protection Agency as an experimental stream to evaluate the impact of a chronic Cu stress on the stream fauna (Winner et al. 1975; Geckler et al. 1976). Except for brief periods during floods, copper was continuously monitored into Shayler Run between stations 1 and 2 at a rate designed to create a concentration of 120 μg Cu/L of stream water. A Cu concentration gradient developed downstream. The biota of Shayler Run was subjected to a rather constant, intermediate to low level Cu stress which had continued for 2½ yr prior to our study.

Elam's Run receives effluent containing Cu, Cr, and Zn from a small metal-plating industry. In contrast to Shayler Run, the concentrations of all three metals are highly variable. At times, concentrations of several mg/L of each metal occur in the stream. At times of high copper concentrations, cyanide may also be present in the mg/L range. The Elam's Run biota had been subjected to these stresses for 8 yr prior to our study.

Methods

Chemical and biological samples were collected from six stations in Shayler Run and five stations in Elam's Run. The stations in Shayler Run were 0.07 km above and 0.2, 0.8, 1.0, 1.2, and 2.6 km below the point of copper discharge. In Elam's Run, sampling stations were 0.4, 0.8, 1.0, 2.1, and 3.4 km downstream from the point of discharge. It was not possible to collect samples upstream from the effluent discharge in Elam's Run because the upstream area was dry during much of the summer. All collecting stations were shallow riffles having a limestone rubble substrate.

Metal concentrations were determined by flame, atomic absorption spectroscopy. Routine water chemistry analyses were performed according to established procedures (American Public Health Association et al. 1975). Benthic samples were collected from Shayler Run during summer 1972 at ~2-wk intervals between July 5 and October 13. Benthic samples were taken from each station in Elam's Run in June, August, and September, 1977, and March, May, and July, 1978. Stations 1 and 5 were also sampled in November 1977. Copper concentrations were determined at each sampling station in Shayler Run when bottom samples were collected (Table 1). Water chemistry, including metal concentrations, was evaluated more frequently in Elam's Run. Fifty-one samples were taken from each station in 1977 and 49 in 1978. In 1978 eight samples were collected from each station (one every 3 h) on June 5, 14, 19; July 6, 13; and August 4.

A Surber sampler, which samples an area of 0.09 m^2, was used in estimating macroinvertebrate densities in Shayler Run. A portable invertebrate box sampler, which samples a surface of 0.1 m^2 (Ellis–Rutter Associates, P.O. Box 394, Douglasville, Pa. 19518), was used in Elam's Run. With both samplers, rubble was removed from within the frame to a tub and scrubbed with a soft brush. Contents of the collecting net were added to the tub and organisms concentrated with a No. 40 sieve in Elam's Run and a No. 30 sieve in Shayler Run. Two samples were always taken from each station. In Shayler Run, organisms were preserved in 70% alcohol and returned to the laboratory for sorting and identification. Elam's Run organisms were sorted alive and chironomid larvae were individually maintained in stream water from the appropriate station. Larvae were fed a suspension of dried dog food. Rearing success was such that most species identifications could be based on larval, pupal, and adult characteristics.

In Elam's Run, emerging insects were also collected with paired emergence traps at each station. These samples were collected during October and November 1977, and during the period from March into October 1978. Traps were emptied at 3- to 4-d intervals; six samples were collected from each station in 1977 and 21 samples in 1978. The traps were modified from a model described by Mundie (Fig. 1.65 in Edmondson and Winberg 1971).

Mean values for both chemical and biological data were compared by a random blocked analysis of variance and Duncan's new multiple-range test (Duncan 1955). Unless otherwise indicated, statements of significant difference are based on accepting $P \leq 0.05$.

TABLE 1. Copper concentrations (μg/L) in Shayler Run, 1972. Distance in km, above or below point of copper introduction in parentheses.

Date	Station 1 (−0.07)	2 (+0.2)	3 (+0.8)	4 (+1)	5 (+1.2)	6 (+2.6)
July 5		123	70	55	47	24
July 21		152	58	46	28	18
July 31		112	55	39	26	16
Aug. 11		108	55	28	20	13
Aug. 30		117	58	38	25	18
Sept. 18		127	58	49	34	21
Oct. 2		106	91	78	71	41
Oct. 13		116	86	81	49	33
Mean	9.0[a]	120	66	52	38	23

[a]Based on 29 samples collected between January 12 and October 10, 1972. Minimum and maximum concentrations were 5 and 24 μg/L. All means are significantly different.

Results

CHEMICAL ENVIRONMENT

Shayler Run — Extensive chemical data for this stream appear in Geckler et al. (1976). Except for copper, variations in chemical conditions were temporal, rather than spatial. Over the 5-yr time span, variation in certain chemical concentrations was as follows: alkalinity (as $CaCO_3$), 126–337 mg/L; hardness (as $CaCO_3$), 185–328 mg/L; orthophosphate, 3.8–8.9 mg/L; and pH, 7.6–8.2. Because the stream was shallow and fast-flowing, low oxygen concentrations did not occur. Compared to Elam's Run (Tables 1 and 3), copper concentrations varied remarkably little within stations and the mean concentrations were significantly different for all stations.

Elam's Run — Unlike Shayler Run, the chemical composition of the water was highly variable in both space and time. In addition to containing fluctuating concentrations of Cu, Cr, and Zn, the metal-plating effluent had a very different chemical matrix from that of the receiving water. This matrix is also quite variable temporally and, since during dry periods the effluent comprises a major fraction of the stream flow, it creates a complex chemical gradient as it mixes downstream with the natural water. The effluent (station 0) was significantly different from all other stations in its conductivity, pH, hardness, and concentrations of orthophosphate, chlorine, and calcium (Table 2). Although downstream trends in chemical characteristics are apparent, mean values for the above parameters were not significantly different at stations 1 through 5. This is partly because the distances between stations were short and partly because of the inherent bias associated with grab samples taken along a chemical gradient which is subjected to erratic slugs of chemicals. Elam's Run, in general, had a higher alkalinity and pH than did Shayler Run. Both streams were phosphate-enriched, Elam's Run from the industrial effluent and Shayler Run from a sewage treatment outfall several kilometres upstream from the study area.

TABLE 2. Conductivity (μmho/cm²) and mean concentrations (mg/L) of six chemicals by station in Elam's Run.[a] Distance downstream in km given in parentheses.

Station	Conductivity	Cl	Hardness	Ca	pH	Alkalinity	Ortho-PO_4
0	1676	0.17	202	95	9.4	285a	23.2
1 (0.4)	937a	0.08a	309b	196a	8.5a	230a	6.4a
2 (0.8)	826a	0.06a	335ab	233a	8.6a	249a	3.7a
3 (1.0)	891a	0.07a	344ab	233a	8.6a	256a	4.0a
4 (2.1)	963a	0.06a	344ab	228a	8.4a	271a	5.5a
5 (3.4)	989a	0.07a	360a	219a	8.4a	275a	5.0a

[a]Means followed by a common letter are not significantly different.

Heavy-metal concentrations exhibited extreme temporal and spatial variability in Elam's Run (Table 3). There were no significant differences in concentrations of any of the metals between times of collection for any of the stations. Differences between stations for individual days agree with differences between stations for the pooled data (Table 3). Concentrations of each metal were always significantly higher in the effluent than at station 1, just 0.4 km downstream. Mean metal concentrations decreased but the measured concentrations at stations 1 through 5 were not significantly different from each other. This reflects the extreme variability in concentrations at each station and the inability to account for "slugs" of metals moving downstream.

Elam's Run was much more heavily polluted in 1978 than in 1977 (Table 3). Mean concentrations of all

TABLE 3. Mean heavy-metal concentrations[a] (μg/L) at six stations in Elam's Run for 1977–1978. (Maximum detected concentrations in parentheses.)

Station	Cu 1977	Cu 1978	Cr 1977	Cr 1978	Zn 1977	Zn 1978
0	114[b] (410)	1250[b] (9590)	70[b] (350)	910[b] (8197)	56[b] (283)	508[b] (2431)
1	63 (220)	336 (2535)	37 (315)	70 (281)	29 (145)	101 (654)
2	31 (100)	237 (1469)	5 (45)	54 (203)	14 (60)	55 (355)
3	40 (175)	221 (1550)	10 (230)	58 (187)	20 (120)	51 (195)
4	32 (135)	87 (827)	1 (15)	15 (52)	13 (72)	24 (66)
5	28 (135)	74 (854)	<1 (5)	2 (17)	16 (75)	24 (107)

[a]Means based on 51 samples collected during 1977 and 45 samples collected during 1978.
[b]Significantly larger than all other means in the column.

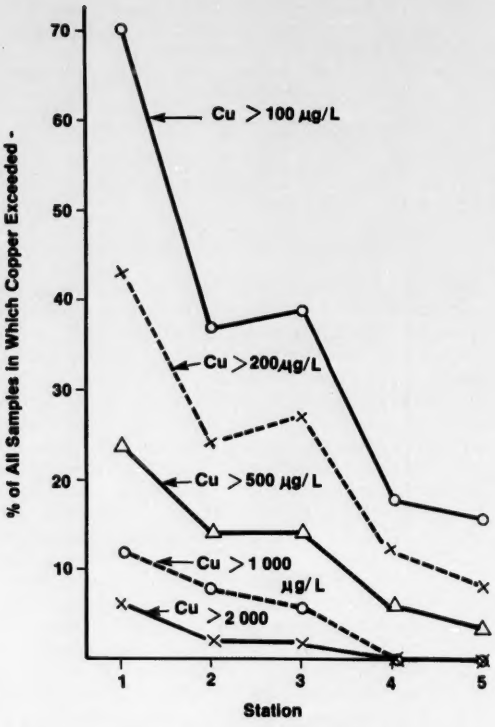

FIG. 1. Percentage of samples containing selected copper concentrations at five stations in Elam's Run, 1978.

three metals were about 10 times greater in 1978 reflecting the industry's increasing its work week from 5 to 6 d and its work days from 16 to 24 h. Chromium concentrations decreased more rapidly downstream than did Zn which, in turn, decreased more rapidly than copper.

The absence of significant differences between mean metal concentrations at the five stations is misleading in evaluating the downstream impact of the effluent on the fauna. The frequency with which biologically damaging concentrations of toxic chemicals occur at each station is probably the most important factor in determining the structure of the invertebrate communities. Of the three metals, copper should have the greatest biological impact since it is the most toxic and reached downstream stations in the highest concentrations. Therefore, we have indicated the frequency with which increasingly damaging concentrations of copper were recorded at each station in Elam's Run during 1978 (Fig. 1). Although metal concentrations were lower in 1977, the same pattern existed. The stations can be placed into three groups in relation to their frequency of exposure to copper concentrations greater

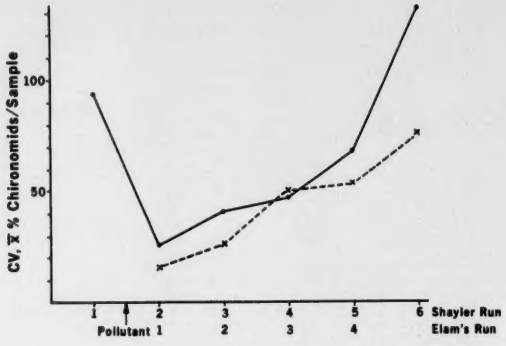

FIG. 2. Changes in the coefficient of variation of mean percentage of chironomids in benthic insect samples taken along heavy-metal concentration gradients in two streams.

than 100, 200, 500, 1000, and 2000 µg/L. Station 1 samples contained copper at each of these concentrations approximately twice as frequently as did samples from stations 2 and 3. Station 4 and 5 samples never contained copper concentrations greater than 1000 µg/L and had a frequency of samples containing copper concentrations greater than 100, 200, and 500 µg/L, approximately one-half that of stations 2 and 3 and one-quarter that of station 1.

COMMUNITY STRUCTURE

Algae — No effort was made to evaluate algal community structure quantitatively but it was evident that stations 1 and 2 in Elam's Run supported luxurious growths of filamentous green algae in the early spring. These attained a maximum density in late April and early May and then suffered a massive die-off. This community is dominated by *Stigeoclonium* sp. at station 1 and by *Cladophora* sp. at station 2.

Noninsect invertebrates — A number of invertebrate taxa which are common in other small southwestern Ohio streams are absent from or rare in Elam's Run and the copper-stressed regions of Shayler Run. These include the bivalve *Pisidium*, gastropod *Physa*, isopod *Lirceus*, flatworm *Dugesia*, and the crayfish, *Orconectes rusticus*.

In contrast, tubificid worms were abundant throughout the study area in Elam's Run. Densities of 1000 to 5000/m^2 occurred in decomposing algal mats at upstream stations and 3000/m^2 occurred in a soft-bottomed pool above station 1 on June 24, 1977. No other macroinvertebrates were found in this latter sample. Dr. Kian Eng Chua of the Royal Ontario Museum kindly examined some of our Elam's Run samples and reported that the species composition was quite consistent between station and dates. The samples contained ~70% *Limnodrilus hoffmeisteri*, 29% *Tubifex tubifex*,

TABLE 4. Structural characteristics of macroinvertebrate communities[a] in Shayler Run, June 5–November 13, 1972.

Station	Insects/ m^2	% chironomids/ sample	% caddisflies/ sample	% mayflies/ sample
1	2448	5a	11ab	42.0a
2	462a	75	6a	2.0b
3	876ab	58	11a	0.3b
4	805ab	41	22b	10.0b
5	1056ab	8a	40	12.0b
6	1652b	3a	21b	36.0a

[a]Means followed by a common letter are not significantly different.

TABLE 5. Structural characteristics of macroinvertebrate communities[a] in Elam's Run, June 1977 through July 1978.

Station	Insects/ m^2	% chironomids/ sample	% caddisflies/ sample
1	1299a	86a	2a
2	1526a	77ab	2a
3	1301a	66abc	14a
4	2498a	60bc	20a
5	2101a	48c	45

[a]Means followed by a common letter are not significantly different.

and 1% *Peloscolex multisetosus*. Tubificids were rare in the Shayler Run samples, probably because all stations had a rock-rubble substrate free of filamentous algae.

Insects — Although insect densities were affected below the point of metal introduction in both streams, they were significantly different only in Shayler Run (Tables 4 and 5). In the latter stream, mean insect density was reduced by 81% just below the point of copper discharge. Although the density had increased fourfold at a point 2.6 km below the outfall, it was still significantly lower than at station 1, upstream from the outfall. Insect densities were more than three times greater at the most heavily polluted station in Elam's Run than at the counterpart station in Shayler Run. Some of this difference can be attributed to the larger numbers of 1st-instar larvae which were retained by the No. 40 sieve used in Elam's Run.

The most striking aspect of insect community structure in both streams is the correlation between chemical stress and the numerical dominance of chironomids (Tables 4 and 5). In Elam's Run, the average percentage of chironomids per sample decreased from 86 at station 1 to 48 at station 5. The correlation coefficient of the percentage of chironomids in samples in relation to the station from which samples were taken was $+0.87$ ($P < 0.05$). In Shayler Run, chironomids averaged only 5% of the insects per sample at station 1 above the copper introduction, increased to 75% of the community at station 2, and then progressively decreased to 3% at station 6. Since there was a single toxicant at rather constant exposures in Shayler Run, it is appropriate to calculate the correlation between mean copper concentration and mean percentage of chironomids in samples from each of the six sampling stations. The correlation coefficient is $+0.93$ ($P < 0.01$).

According to Barber and Kevern (1974), it is necessary to use a mesh size of 0.20 to 0.24 mm to guarantee the retention of 1st-instar larvae of all invertebrates. Their data indicate that a considerable fraction of the smaller chironomid individuals should have been lost even through the No. 40 sieve used in Elam's Run. In spite of this, a sufficient fraction of the chironomid community was retained to document the relationship between chironomid dominance and heavy-metal pollution in both streams. Use of a finer mesh might strengthen the relationship and/or reduce some of the variability, but in view of the tremendous increase in sorting time, the added information is probably not worth the extra effort in routine surveys of lotic habitats.

There was also a pattern of temporal and spatial variability in community structure, as measured by relative numerical dominance of chironomids, which is consistent for the two streams. Community structure was progressively less predictable along a gradient of decreasing heavy-metal concentrations. We have used the coefficient of variation (CV) of percentage of chironomids in samples from each station as the index of variability (Fig. 2). In Shayler Run, it decreased from 94.2 just above the point of copper introduction to 26.4 just below, and then progressively increased to 132.0 at station 6. In Elam's Run, it increased from 16.0 at station 1 to 75 at station 5.

Fifty-seven species of chironomids representing 26 genera were collected from a 3-km stretch of Elam's Run during our study (Table 6). This table points out the danger of using even a generic level of identification in calculating diversity indices. One of the 26 genera was represented by eight species and 13 were represented by more than one species. Six species of *Cricotopus* and seven species of *Pentaneura* were collected from station 4 alone.

Although the relative numbers of chironomids decreased at stations downstream from the outfall, the number of species increased from 15 at station 1 to 39 at stations 4 and 5 (Table 6). This increase in diversity involves an increase in, but not a substitution of, species. Of the 15 species from station 1, 13 were also collected at station 5. *Cricotopus bicinctus* and *C. infuscatus* were either the dominant or codominant species at stations 1, 2, 3, and 4, comprising 92, 77, 77, and 41% of all chironomids identified from samples at those stations, respectively. Although both species were collected at station 5, they comprised only 20% of all individuals. In 1977, when metal concentrations were lower, the two species of *Cricotopus* constituted only

TABLE 6. Check list of Elam's Run midges by station.

Species	Station[a] 1	2	3	4	5
Cricotopus exilis Johannsen	E			E	E
C. infuscatus (Malloch)	X	X	X	X	X
C. bicinctus (Meigen)	X	X	X	X	E
Pentaneura sinuosa[b] (Coquillett)	X	X	X	X	E
P. melanops[b] (Meigen)	X	X	B	X	X
Polypedilum convictum (Walker)	E	X	E	E	E
Natarsia baltimoreus[b] (Macquart)	B	B	B	B	B
Procladius culiciformis[b] (Linnaeus)	B	E	B	B	E
Corynoneura nr. *scutellata* Winnertz	E	X		E	E
Metriocnemus lundbeckii Johannsen	E		B	X	E
Larsia planensis[b] (Johannsen)		E			
Orchocladius dubitatus Johannsen	B	X		X	X
Chironomus attenuatus Walker	B		X	X	B
Paratendipes albimanus (Meigen)	X				B
Psectrotanypus dyari[b] (Coquillett)	B	B	B	E	
Chironomus riparius Meigen	B			E	
Dicrotendipes neomodestus (Malloch)		B			
Diplocladius cultriger Kieffer		B		B	
Pentaneura bifasciata[b] (Coquillett)		B		B	
Larsia decolorata[b] (Malloch)		E	X	B	
Cricotopus trifasciatus (Panzer)		E	B	B	
Tanytarsus neoflavellus Malloch		X	E	E	X
Micropsectra deflecta (Johannsen)		E	B	X	X
Cricotopus varipes Coquillett		E	B	E	E
Pentaneura flavifrons[b] (Johannsen)		E		E	B
Thienemanniella nr. *similis* (Malloch)		E		E	B
Phaenopsectra flavipes (Meigen)		E			E
Eukiefferiella sordens (Johannsen)		X	E	X	E
Cryptochironomus fulvus[b] (Johannsen)		B	E	X	X
Eukiefferiella brevinervis (Malloch)		B	B	E	E
Orchocladius obumbratus Johannsen		X	X	X	X
Cryptochironomus digitatus[b] (Malloch)		B			X
Stictochironomus flavicingula (Walker)		B		B	X
Parachironomus tenuicaudatus (Malloch)			B		
Orthocladius johannseni Sublette			B	B	
Cricotopus slossonae Malloch			E	E	
Tanytarsus exiguus Johannsen			B	B	
Pentaneura currani[b] (Beck and Beck)				E	
Dicrotendipes fumidus (Johannsen)			B		B
Pentaneura pilosella[b] (Loew)				E	
Phaenopsectra obediens (Johannsen)				B	
Pentaneura fimbriata[b] (Walker)				E	
Microtendipes pallidus (Johannsen)				E	
Cryptotendipes pseudotener (Goetghebuer)				E	
Trichocladius nitidus (Malloch)				X	X
Tanytarsus dissimilis Johannsen				B	B
Polypedilum halterale (Coquillett)					E
Metriocnemus aequalis Johannsen					E
M. exagitans Johannsen					E
M. atratulus (Zetterstedt)					E
Ablabesmyia monilis[b] (Linnaeus)					E
Orchocladius stamfordi (Johannsen)					B
Tanytarsus viridiventris Malloch					E
Pentaneura cornuticaudata[b] (Walley)					B
Diamesa nivoriunda (Fitch)					B
Micropsectra dives (Johannsen)					B
Polypedilum scalaenum (Schrank)					E

[a]X = collected in both emergence and box samplers; B = collected only in box samplers; E = collected only in emergence samplers.

[b]Predaceous larvae.

10% of all midges collected at station 5. Of the 39 species collected from station 5, 26 were never collected from station 1.

There is also a downstream increase in the number of unique species collected per station. Station 4 and 5 samples contained the same number of species, but 11 unique species were collected from station 5 in contrast to only 6 unique species at station 4. No unique species were collected from station 1, only two unique species were collected from station 2, and only one unique species was collected from station 3 (Table 6).

Some species of caddisflies are relatively tolerant to heavy-metal pollution. Although caddisflies were virtually absent from stations 1 and 2 in Elam's Run (Table 5), they comprised 14, 20, and 45% of all insects collected from stations 3, 4, and 5. These stations are subjected to a chronic, moderate level of pollution and to occasional slugs of very high concentrations of metals (Table 3 and Fig. 1). The spatial distribution of caddisflies in Shayler Run agrees with the pattern of Elam's Run (Table 4). This taxon was relatively and absolutely more abundant at stations receiving moderate copper pollution than it was at the unpolluted station.

In Shayler Run, mayflies were the last of the insects to recover downstream from the copper stress (Table 4). Although chironomid, beetle, caddisfly, and damselfly immatures all exhibited higher relative and absolute densities at intermediate copper concentrations than in the unpolluted portion of the stream (Winner et al. 1975), those of mayflies did not. In Elam's Run, mayflies comprised less than 1% of all insects collected at station 5, farthest downstream from the point of effluent discharge.

Discussion

ALGAE

The dominance of *Stigeoclonium* at station 1 in Elam's Run is in agreement with reports in the literature that some species in this genus are among the most metal-tolerant of the lotic algae (Butcher 1955; Surber 1959; Whitton 1970). The presence of *Cladophora* at station 2, however, contradicts the generalization of Whitton (1970) that "an abundant growth of *Cladophora* in freshwater indicates that these waters are not subject to repeated pollution by heavy metals in solution."

NONINSECT INVERTEBRATES

The occurrence of *L. hoffmeisteri*, *T. tubifex*, and *Peloscolex multisetosus* in the most polluted areas of Elam's Run indicates that these species are very tolerant of high concentrations of Cu, Cr, and Zn. We have found no literature substantiation for heavy-metal tolerance by *P. multisetosus*, but Popović and Popović (1977) demonstrated that *T. tubifex* can tolerate high concentrations of Cu, Cr, and Zn under laboratory condi-

tions, and Pagel and Wade (1976) reported that *L. hoffmeisteri* replaced another oligochaete (*P. ferox*) subsequent to applications of $CuSO_4$ in Lake Champlain.

King and Ball (1964) suggested that tubificid worms are more resistant to pollutants, including heavy metals, than are aquatic insects, and that the ratio of tubificids to insects can be used as a pollution index. The data for Elam's Run clearly indicate that some species of both insects and tubificids can thrive in lotic environments that are grossly polluted with heavy metals. Soft-bottomed pools in the upper reaches of Elam's Run supported high oligochaete densities and few insects but adjacent rubble-bottomed riffles supported high densities of chironomids. The relative dominance of the two taxa was obviously controlled by factors other than heavy-metal tolerance such as by differences in substrate and available food resources. This is further supported by the fact that oligochaetes were very abundant in the rock-rubble riffles during brief periods when the rubble was covered with decomposing mats of filamentous algae.

Insect Community Structure

Faunal lists from benthic surveys of small, unpolluted streams in Europe, Great Britain, and North America indicate that chironomids comprise no more than 20% of the total insect community of riffle habitats in which the rock substrate is free of filamentous algae or aquatic mosses (Wynes 1979; Cummins et al. 1966; Morgan and Egglishaw 1965; Egglishaw 1964; Hynes 1961; O'Connell and Campbell 1953; Percival and Whitehead 1929). We have been able to find only one reference from which we can calculate the fraction of an insect community comprised by chironomids in a metal-polluted stream. Surber (1959) presented tabular data on the invertebrate fauna above and below the discharge of an electroplating effluent into the Clinton River, Michigan. Although he did not call attention to the fact, chironomids increased from 7.5% of the total individuals collected from above to 96% below the point of effluent discharge.

The fraction of a community composed of chironomids becomes more unpredictable as the severity of a heavy-metal stress decreases. We interpret this to mean that a continuous, severe chemical stress swamps the spatial and temporal heterogeneity of other environmental factors. This results in a consistently harsh environment which is predictably dominated by chironomids. As the severity of the chemical stress decreases, substrate and seasonal variability begin to affect community composition and the chironomid fraction of the community becomes more variable in space and time. Chironomids may still numerically dominate some samples, but with decreasing regularity. Community structure can therefore be characterized by fewer samples in polluted habitats than in unpolluted habitats which exhibit comparable spatial and temporal heterogeneity.

It is difficult to relate the species composition of chironomid communities in Elam's Run to those of other streams because there are few published records of the number of chironomid species occurring in small streams. Pennak (1978) states that highly productive streams may support more than 30 species of chironomids. Thienemann (1941) recorded 44 species from Lappland streams. Lehmann (1971) recorded 246 species of chironomids from the Fulda in Germany. This study, however, encompassed 220 km of a large river system. Coffman (1973) reported 143 chironomid taxa from a 1-km section of a small woodland stream in Pennsylvania. Although both studies involved small streams in the deciduous forest zone of North America, it is difficult to compare our data with Coffman's for several reasons. His results are based on collections of pupal exuviae, many of which were not identified to species, or, in some cases, even to genus. Therefore, the number of species is really unknown. It is also impossible to determine the microhabitat from which the pupae originated, while all of the Elam's Run species are known to have developed in shallow riffle areas.

We have been able to find information relating to the heavy-metal tolerance of only one species of midge. Surber (1959) found *C. bicinctus* in a river having concentrations of Cr^{+6}, Cu, and cyanide up to 25.0, 212.0, and 3.3 mg/L. Although a heavy-metal stress, like other chemical stresses, will decrease the number of chironomid species within a community, data are presently insufficient to predict the species composition to be expected in particular concentrations or combinations of metals. The data in Table 6 are a start in that they document a surprisingly large number of chironomid species which can tolerate long-term exposure to a multiple metal stress of sufficient magnitude to eliminate essentially most other insect families.

Winberg (1978) has proposed a pollution index which assumes that clean waters are dominated by larvae in the subfamily Orthocladiinae and polluted waters by larvae in the subfamily Tanypodinae. The kinds of pollutants to which the index is sensitive are not specified. If one considers the numbers of individuals in each subfamily, this index is not valid in relation to heavy-metal pollution in Elam's Run. The two dominant species in the most grossly polluted regions of Elam's Run, *C. bicinctus* and *C. infuscatus*, are both members of the Orthocladiinae and 40% of the species collected from station 1 belong to that subfamily (Table 6).

Although predaceous larvae of the Tanypodinae are not numerically dominant at the most polluted station, they do comprise a larger fraction of the species pool there than at the least polluted station (Table 6). Thirty-three percent of the larvae collected at station 1 belong to taxa which are considered predaceous, whereas only 23% of the larvae collected from station 5 belong to these taxa. This decrease in numbers of nonpredaceous species at the most polluted station may be related to other factors such as changes in the kinds of food avail-

able. It is obvious, however, that many species of predaceous midges are no more sensitive to heavy-metal pollution than are the herbivorous or detritivorous species.

Two other taxa of predaceous insects were collected as far upstream as station 2 in Elam's Run. The neuropteran, *Sialis* sp., occurred as frequently in samples from upstream as it did in samples from downstream. Although only infrequently collected, predaceous damselfly naiads were observed in large schools in a pool just below station 2 and were collected in greatest abundance at station 2. In Shayler Run, damselfly naiads attained their greatest relative and absolute densities at station 3 (Winner et al. 1975).

There are some data in the literature which support our findings on the relative sensitivity of caddisflies and mayflies to heavy metals. Sprague et al. (1965) indicated that caddisflies were 2.5 times as abundant in sections of the Miramichi River polluted with copper and zinc as they were in unpolluted sections. They also indicated that mayflies were very sensitive to this copper–zinc pollution. Södergren (1976) reported that mayflies were the most sensitive of invertebrate taxa to cobalt–nickel pollution in a salmon river in northern Sweden.

The relative rarity of stoneflies in Shayler Run and Elam's Run is probably not related to heavy-metal pollution. Stoneflies were uncommon at the control station in Shayler Run and are minor components of small limestone streams in the general region.

In summary, the impact of a heavy-metal stress on the macroinvertebrate community structure in Shayler Run and Elam's Run is remarkably similar in spite of the fact that the former stream was subjected to a single toxicant at relatively constant concentrations and the latter stream was subjected to multiple toxicants at higher and highly variable concentrations. It appears that a continuous, low-level stress has a gross biological impact comparable to that of intermittent insults of much greater intensity. The consistent relationship between intensity of chemical stress and the ratio of chironomids to total insects in the community suggests that this ratio should be evaluated for other kinds of chemical stresses. Furthermore, we suggest that the relative numbers of chironomids, caddisflies, and mayflies, may indicate various degrees of heavy-metal stress with only chironomids surviving in the most polluted waters, chironomids and caddisflies both being abundant in areas of moderate pollution, and caddisflies and mayflies being abundant in waters subject to little toxic stress. It is important to remember, however, that individual samples from unpolluted waters may contain a high percentage of chironomids. It is necessary to take multiple samples throughout the seasons if community structure is to be adequately characterized.

Acknowledgments

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American Public Health Association, American Water Works Association, and Water Pollution Control Federation. 1975. Standard methods for the examination of water and wastewater. 14th ed. Washington, D.C. 1193 p.

Barber, W. E., and N. R. Kevern. 1974. Seasonal variation of sieving efficiency in a lotic habitat. Freshwater Biol. 4: 293–300.

Butcher, R. W. 1955. Relation between the biology and the polluted condition of the Trent. Verh. Int. Verein. Limnol. 12: 823–827.

Clarke, R. McV. 1974. A summary of toxicity information for major effluent components from inorganic chemical industries. Tech. Rep. Ser. No. CEN/T-74-9. Environ. Can., Winnipeg. 27 p.

Coffman, W. P. 1973. Energy flow in a woodland stream ecosystem: II. The taxonomic composition and phenology of the Chironomidae as determined by the collection of pupal exuviae. Arch. Hydrobiol. 71: 281–322.

Cummins, K. W., W. P. Coffman, and P. A. Roff. 1966. Trophic relations in a small woodland stream. Verh. Int. Verein. Limnol. 16: 627–638.

Duncan, D. B. 1955. Multiple range and multiple F tests. Biometrics 11: 1–42.

Edmondson, W. T., and G. G. Winberg [ed.]. 1971. IPB Handbook 17, A Manual on methods for the assessment of secondary productivity in freshwaters. Blackwell Sci. Publ., Oxford and Edinburgh. 358 p.

Egglishaw, H. J. 1964. The distributional relationship between the bottom fauna and plant detritus in streams. J. Anim. Ecol. 33: 463–476.

Geckler, J. R., W. B. Horning, T. M. Neiheisel, Q. H. Pickering, and E. L. Robinson. 1976. Validity of laboratory tests for predicting copper toxicity in streams. Ecol. Res. Ser. Environ. Prot. Agency 600/3-76-116. Environ. Res. Lab., U.S. Environ. Prot. Agency, Duluth, MN. 192 p.

Hynes, H. B. N. 1961. The invertebrate fauna of a Welsh mountain stream. Arch. Hydrobiol. 57: 344–388.

King, D. L., and R. C. Ball. 1964. A quantitative biological measure of stream pollution. J. Water Pollut. Control Fed. 36: 650–653.

Lehmann, J. 1971. Die Chironomidae der Fulda. Arch. Hydrobiol. Suppl. 37: 466–555.

Morgan, N. C., and H. J. Egglishaw. 1965. A survey of the bottom fauna of streams in the Scottish Highlands Part I. Composition of the fauna. Hydrobiology 25: 181–211.

O'Connell, T. R., and R. S. Campbell. 1953. The benthos of the Black River and Clearwater Lake, Missouri. Univ. MO. Stud. 26: 25–41.

Pagel, C., and C. Wade. 1976. Benthic changes in a bay receiving annual copper sulfate applications in Lake Champlain. Abstr., 24th Ann. Meet. N. Am. Benth. Soc., LaCrosse, Wis.

Pennak, R. W. 1978. Freshwater invertebrates of the United States. 2nd ed. John Wiley Sons, N.Y. and Toronto. 803 p.

Percival, E., and H. Whitehead. 1929. A quantitative study of the fauna in some types of stream bed. J. Ecol. 17: 282–314. (As cited in Hynes (1970))

POPOVIĆ, I. B., AND M. POPOVIĆ. 1977. Effects of heavy metals on survival and respiration rate of tubificid worms: Part I—Effects on survival. Environ. Pollut. 13: 65–98.

SÖDERGREN, S. 1976. Ecological effects of heavy metal discharge in a salmon river. Inst. Freshwater Res. Drottingholm 55: 91–131.

SPRAGUE, J. B., P. F. ELSON, AND R. L. SAUNDERS. 1965. Sublethal copper–zinc pollution in a salmon river—A field and laboratory study. Air Water Pollut. 9: 531–543.

SURBER, E. W. 1959. *Cricotopus bicinctus*, a midgefly resistant to electroplating wastes. Trans. Am. Fish. Soc. 89: 111–116.

THIENEMANN, A. 1941. Lapplandische Chironomiden und ihre Wohngewasser. Arch. Hydrobiol. Suppl. 17: 1–253.

U.S. ENVIRONMENTAL PROTECTION AGENCY. 1976. Qualitative criteria for water (prepublication copy). Washington, D.C. 501 p.

WHITTON, B. A. 1970. Toxicity of heavy metals to freshwater algae, a review. Phykos 9: 116–125.

WINBERG, G. G. 1978. Experimental application of various systems of biological indication of water pollution, p. 14–149. *In* D. I. Mount [ed.] Vol. I, Proc. of 1st and 2nd USA–USSR Symp. on effects of pollutants upon aquatic ecosystems, Environ. Res. Lab., U.S. Environ. Prot. Agency, Duluth, MN.

WINNER, R. W., J. SCOTT VAN DYKE, N. CARIS, AND M. P. FARRELL. 1975. Responses of the macroinvertebrate fauna to a copper gradient in an experimentally-polluted stream. Verh. Int. Verein. Limnol. 19: 2121–2127.

WYNES, D. L. 1979. Predator–prey relationships in the riffle fish community of the Little Miami River, Ohio. Ph.D. dissertation, Miami Univ., Oxford, Ohio.

Effects of Tubificid Worms on Denitrification and Nitrification in Stream Sediment[1]

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CHATARPAUL, L., J. B. ROBINSON, AND N. K. KAUSHIK. 1980. Effects of tubificid worms on denitrification and nitrification in stream sediment. Can. J. Fish. Aquat. Sci. 37: 656–663.

Labeled nitrate-N in the form of $K^{15}NO_3$ was used to determine the effects of the tubificid oligochaetes, *Tubifex tubifex* and *Limnodrilus hoffmeisteri*, on the N transformation processes in stream sediment contained in laboratory columns. Denitrification and nitrification occurred simultaneously and were enhanced significantly in the presence of the worms. The denitrification rate over 48 d at 15°C in the sediment that contained the worms was 90 mg $N/m^2 \cdot d^{-1}$; the simultaneous rate of nitrification was 69 mg $N/m^2 \cdot d^{-1}$. The corresponding rates for denitrification and nitrification in sediment without worms were 50 mg $N/m^2 \cdot d^{-1}$ and 29 mg $N/m^2 \cdot d^{-1}$. The rate of CO_2 evolution from sediment containing worms and overlain with nitrate-N solution was about 21% greater than that of sediment with worms overlain with distilled water. It was concluded from experiments using chloride as tracer that denitrification could be enhanced by the worms at least in part as a result of accelerated movement of nitrate-N into the sediment. However, when a column of glass beads was used as substrate for worms, and with no organic matter present, the concentration of nitrate in an overlying solution declined at an appreciable rate. This suggested that denitrification occurred in or on the worms, a possibility supported by the observation that denitrifying bacteria could be isolated from both the exterior and the gut contents of the worms.

Key words: tubificid worms, stream nutrient cycling, denitrification, nitrogen cycling

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Les effets des oligochètes tubificides, *Tubifex tubifex* et *Limnodrilus hoffmeisteri*, sur les processus de transformation de N dans un sédiment lotique renfermé dans des colonnes de laboratoire, ont été déterminés en utilisant de l'azote nitrique sous forme de $K^{15}NO_3$. La dénitrification et la nitrification se produisent simultanément et sont intensifiées de façon marquée en présence de vers. Le taux de dénitrification sur une période de 48 jours à 15°C dans le sédiment contenant des vers est de 90 mg $N/m^2 \cdot d^{-1}$; le taux de nitrification coïncident est de 69 mg $N/m^2 \cdot d^{-1}$. Pour les sédiments dépourvus de vers, les taux correspondants de dénitrification et de nitrification sont de 50 mg $N/m^2 \cdot d^{-1}$ et 29 mg $N/m^2 \cdot d^{-1}$. Le taux d'évolution du CO_2 du sédiment contenant des vers et recouvert d'une solution d'azote nitrique est d'environ 21% supérieur à celui du sédiment avec vers mais recouvert d'eau distillée. Des essais avec chlorure comme marqueur permettent de conclure que la dénitrification pourrait être accentuée par les vers comme résultat, au moins en partie, du mouvement accéléré de l'azote nitrique dans le sédiment. Cependant, quand on utilise une colonne de billes de verre comme substrat pour les vers, sans matière organique, la concentration de nitrate dans une solution sus-jacente diminue à un taux appréciable. Ceci donne à croire que la dénitrification se produit sur ou dans les vers, une possibilité appuyée par l'observation que des bactéries dénitrifiantes peuvent être isolées à la fois de l'extérieur et du contenu du tractus digestif des vers.

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RECENTLY attention has been drawn to the possibility that denitrification in sediments results in losses of nitrogen from stream water, despite the fact that the water in streams is usually well oxygenated (Kaushik et al. 1975; Toms et al. 1975; Kaushik and Robinson 1976; Van Kessel 1977a; Robinson et al. 1979; Chatarpaul and Robinson 1979; Hill 1979). In this regard effects of such factors as pH, temperature, oxygen and nitrate concentrations, and sediment depth have been studied (Toms et al. 1975; Van Kessel 1977b; Sain et al. 1977). However, the role of benthic animals in stream

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nitrogen cycling has received little attention.

Recently, Chatarpaul et al. (1979) reported that the presence of tubificid worms, *Limnodrilus hoffmeisteri* and *Tubifex tubifex*, appeared to increase the rate of nitrification and also the rate at which nitrate-N disappeared from aerated water overlying Canagagigue Creek sediment. They postulated that the worms were able to enhance denitrification by increasing diffusion and mechanical transfer of nitrate-N to sites for denitrification in the sediment. Because the processes of denitrification and nitrification proceed simultaneously, it is difficult to quantify the effects of the worms on these processes without the use of tracers such as ^{15}N. This paper presents the results of a study using labeled nitrate-N designed to determine unequivocally whether or not tubificid worms influence denitrification and nitrification and to elucidate the mechanism of these effects.

Materials and Methods

Experiments were carried out in triplicate Plexiglas columns (60 cm × 6.4 cm OD) in which stream sediment, taken from Canagagigue Creek, a tributary of the Grand River, Ontario, was overlain with continuously aerated nitrate solution. Details of sampling sites, sediment collection, and preparation of columns have been given by Chatarpaul et al. (1979). The sediment, which was well mixed, was 10 cm deep in the columns, and the volume of nitrate solution was 500 mL. For aerating the solution, a gas mixture of 21% oxygen and 79% helium was introduced by a sparger located about 1 cm above the sediment–water interface. In those experiments involving nitrogen budgets, vials containing 2% boric acid were placed in the gas outflow line from each column to monitor volatile ammonia. Occasionally these vials were used as traps for CO_2 by filling them with 2.5 mL, 1 N NaOH and a filter paper wick. In the latter case, the vials were titrated with HCl solution following addition of $BaCl_2$ solution (Sain and Broadbent 1977).

The tubificid worms *L. hoffmeisteri* and *T. tubifex* were obtained from Toronto Harbour near Hanlan's Point. When required, 30 or 40 worms were added to each column to give an approximate density of 12 000 worms/m^2 or 16 000 worms/m^2 sediment surface, a density often encountered in nature.

For ^{15}N experiments, 500 mL of solution containing 9.1 mg/L nitrate-N as $K^{15}NO_3$ (90 at.% ^{15}N) was used in each column. At selected sampling times, triplicate columns were "sacrificed" and the water overlying the sediment was drawn off by suction. Analyses for ammonium-N, nitrate-N, and organic-N were performed on both the water and the wet sediment according to the standard procedures of Bremner (1965a, b) and APHA (1971) as modified by Chatarpaul and Robinson (1979). All N samples thus quantified were further processed for determination of ^{15}N abundances. The ^{15}N isotope ratio analyses were performed on an optical emission spectrometer (Jasco NIA-I ^{15}N Analyser). Details of sample preparation and analytical procedures are given by Chatarpaul and Robinson (1979). Briefly, this method involves placing a sample (containing about 10 μg of N) along with copper oxide, cuprox–platinum catalyst (Coleman Co., Illinois), and an alumina–calcium oxide briquette into a Pyrex discharge tube. The discharge tube is evacuated, sealed, and combusted at 550°C to convert all nitrogen in the sample to N_2. When excited, the N_2 molecules in the discharge tube emit light at 2977, 2983, and 2989 Å corresponding to $^{28}N_2$, $^{29}N_2$, and $^{30}N_2$, respectively. These signals are then resolved and recorded by the ^{15}N Analyser and used to quantify ^{15}N abundances according to the method of Fiedler and Proksch (1975). For calculation of absolute quantities of excess ^{15}N in each sample, the following formula was used:

$$^{15}N(mg) = \frac{\text{at.\% excess } ^{15}N \times \text{quantity of N(mg)}}{100}.$$

To determine whether the worms increased the movement of solute into the sediment, columns with and without worms were overlain with a solution containing 100 mg/L of chloride (KCl). These columns were incubated at room temperature (22°C) for 13 days and analyses for chloride concentration in the supernatant were performed at day 0, 3, 6, 10, and 13 of incubation. To measure the effect of the worms on the redox profile of the sediment, redox probes were installed in the sediment at depths of 1.0, 2.5, 5.0, and 10.0 cm below the sediment–water interface according to the method described by Robinson et al. (1978).

To test the possibility that denitrification might be associated with the worms themselves, nine Pyrex tubes, 200 mm × 30 mm, were packed to a depth of 4 cm with inert micro-glass beads of size 300–500 μm (Catophote Corp. No. 608), which were then washed and boiled in distilled water. The overlying water was then drawn off by suction. Six tubes received 50 mL of nitrate-N solution (9.22 mg/L) and three of these each received 40 worms that had been washed to remove adhering mud particles. The remaining three tubes received 50 mL of distilled water and also 40 worms each. The overlying solution in each tube was gently aerated.

Estimations of denitrifiers and nitrate reducers were made according to an improved most probable number (MPN) method described by Focht and Joseph (1973) and utilizing Difco nitrate broth. The microflora of the outside surface of the worms was removed by a method described by Wavre and Brinkhurst (1971). After the worms were rinsed to remove fecal matter, they were individually washed in five changes of sterile water. The five washings were pooled to make up 10 mL and used in making 10-fold serial dilutions. To estimate internal populations, the washed worms were then macerated in 2.5 mL of sterile water by using a micro-homogenizer (Virtis Co.) and after a brief settling period, the supernatant was suspended in 10 mL of sterile water. This suspension was then used in making 10-fold serial dilutions. Five tubes per dilution were employed and all tubes were incubated at 30°C for 14 d before being tested for the presence of nitrate and nitrite.

Results

Typical analyses for samples of Canagagigue sediment were pH 7.2; ammonia-N, 47.5 ± 2.3 mg/L; organic-N, 0.24 ± 0.01%; combustible matter, 6.40 ± 0.01%; C/N ratio, 13:1; neither nitrate-N nor nitrite-N was detected in the sediment.

In the first experiment, columns with labeled nitrate-N were incubated at 15°C for 48 days. One set of columns received 30 oligochaete worms each. At days 0,

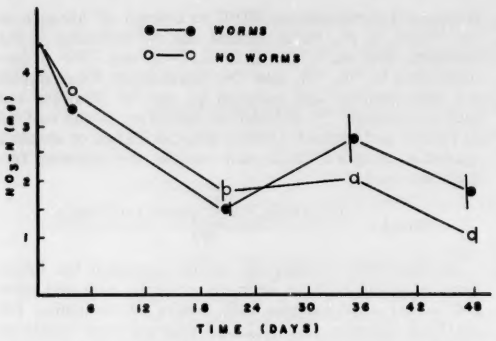

FIG. 1. Changes in concentration of nitrate-N (total of labeled and unlabeled) in columns incubated at 15°C for 48 d with and without oligochaete worms. Values are expressed as mean ± SD.

4, 21, 35, and 48 of the incubation period, triplicate columns with and without worms were "sacrificed" and both overlying water and sediment from each column analysed for organic-N, ammonium-N, and nitrate- and nitrite-N. No N was detected in the vials designed to trap volatile ammonia-N. The respective quantities of ^{15}N excess were then determined in each of the nitrogen fractions. This approach facilitated budgeting for labeled N in the inorganic-and organic-N pools. The ^{15}N not accounted for in this manner was presumed to have been lost from the columns as gaseous products of denitrification.

The quantities of nitrate-N in the water above sediments at different time periods are shown in Fig. 1. The loss of nitrate-N from columns with worms in the first 21 d was slightly more rapid than from those without. However, this difference, unlike that reported earlier (Chatarpaul et al. 1979), was not significant. The reason for the present observation was, as shown later, the more rapid nitrification (and concurrent denitrification) in the columns with worms. An increase in nitrate-N in the water was recorded in both treatments after 21 days, but this increase was larger in the columns with worms. Analyses for other forms of nitrogen in the water and sediment fractions (Table 1) showed little change in the concentration of ammonium-and organic-N while nitrate-N was never detected in the sediment fraction of either treatment.

Excess ^{15}N (above a background level of 0.365 ± 0.005 at.%) was determined in all the above samples. In Fig. 2 the changes in total ^{15}N excess (total from all forms of N) in columns in both water and sediment, with and without worms, are compared. An analysis of variance (ANOVA) showed that the columns containing worms lost labeled N at a rate significantly greater ($P < 0.01$) than did the controls. The distribution of excess ^{15}N in all nitrogen species is shown in Table 2. Only a small quantity of the added labeled N was present in the ammonium- and organic-N forms.

The ^{15}N abundance of the nitrate-N in the solution above sediment in the columns changed from about 90 at.% initially to about 15 at.% at the end of the experiment. This was an indication that substantial nitrate production occurred. The level of isotope dilution at each sampling time could be calculated, and using this information the quantity of nitrate-N produced in the columns was calculated from the derived equation

$$(1)\quad N_g = N_1 - N_o + \frac{A_o N_o - A_1 N_1}{\frac{A_o + A_1}{2}}$$

TABLE 1. The distribution of NH_4-N, NO_3-N, and organic-N in the water and sediment fractions of the columns during the 48-d incubation period at 15°C with and without oligochaete worms. Values are expressed in milligrams as means ± SD except organic-N (%).

		Incubation period (days)									
		0		4		21		35		48	
Column fraction	Nitrogen species	No worms	Worms	No worms	Worms	No worms	Worms	No worms	Worms	No worms	Worms
Water	NH_4-N	0	0	0.49 ±0.12	0.65 ±0.10	0.52 ±0.40	0.44 ±0.57	0	0	0	0
	NO_3-N	4.55 ±0.05	4.55 ±0.05	3.64 ±0.07	3.28 ±0.01	1.79 ±0.41	1.52 ±0.18	2.14 ±0.16	2.74 ±0.53	1.04 ±0.17	1.76 ±0.61
	Organic-N	0	0	0.21 ±0.01	0.22 ±0.02	0.39 ±0.16	0.37 ±0.03	0.29 ±0.04	0.34 ±0.01	0.49 ±0.21	0.36 ±0.02
Sediment	NH_4-N	7.70 ±0.10	7.70 ±0.10	7.68 ±0.22	7.94 ±0.21	8.40 ±0.55	7.80 ±0.26	8.25 ±0.32	8.33 ±0.21	8.23 ±0.29	9.29 ±0.00
	NO_3-N	0	0	0	0	0	0	0	0	0	0
	Organic-N (soluble)	0.76 ±0.03	0.76 ±0.03	0.80 ±0.08	0.84 ±0.11	0.66 ±0.12	0.73 ±0.10	0.60 ±0.20	1.06 ±0.50	1.25 ±0.03	1.88 ±0.12
	Organic-N (%)	0.245 ±0.005	0.245 ±0.005	0.240 ±0.006	0.237 ±0.015	0.233 ±0.008	0.229 ±0.019	0.228 ±0.014	0.230 ±0.024	0.228 ±0.012	0.227 ±0.021

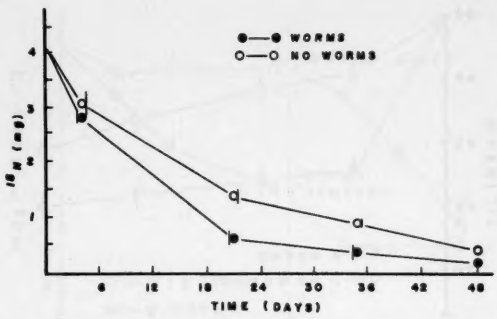

FIG. 2. Changes in total excess ^{15}N (total from all N fractions) in water and sediment phases of columns incubated at 15°C for 48 d with and without oligochaete worms. Values are expressed as mean ± SD.

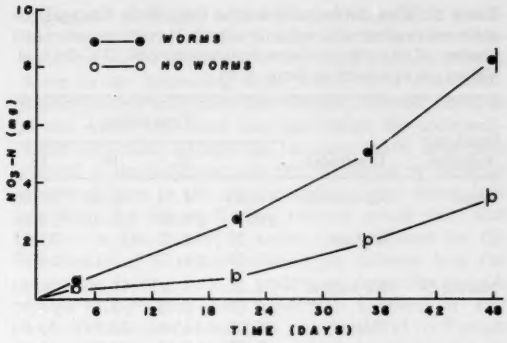

FIG. 3. Estimated production of unlabeled nitrate-N in sediment of columns incubated at 15°C for 48 d with and without oligochaete worms. Values are expressed as mean ± SD.

where N_g = quantity of N gained through nitrification, A_o = the at.% excess ^{15}N in the initial fraction, A_1 = the at.% excess ^{15}N in the final fraction, N_o = the quantity of N in the initial fraction, N_1 = the quantity of N in the final fraction. The production of nitrate-N in the columns is shown in Fig. 3. Nitrate-N production in the columns containing worms was significantly greater ($P < 0.01$) than that in the control columns. In the presence of worms, nitrate was produced in amounts which when averaged over a period of 48 d yielded a rate of 69 mg/m$^2 \cdot$d^{-1}, while the corresponding value for the columns without worms was 29 mg/m$^2 \cdot$d^{-1}. Therefore, in estimating the actual amount of nitrate-N lost via denitrification, that which was added via nitrification must be considered. For example, in each column 4.55 mg of nitrate-N was added at the beginning of the experiment and on the average the solution in the columns with worms received another 8.20 mg of nitrate-N because of nitrification (Fig. 3). Thus each of the columns with worms received a total of 12.75 mg of nitrate-N. At the end of the experiment the solution contained about 2 mg of nitrate-N. On this basis a column with worms lost 10.75 mg of nitrate-N in 48 d or 90 mg/m$^2 \cdot$d^{-1}. The corresponding value for columns without worms was 50 mg N/m$^2 \cdot$d^{-1}. The worms thus increased the rate of denitrification by about 80%.

The second experiment was designed to determine

TABLE 2. The distribution of excess ^{15}N (NH_4-N, NO_3-N, and organic-N) in water and sediment fractions of the columns during the 48-d incubation period at 15°C with and without oligochaete worms. Values are expressed in milligrams as mean ± SD.

		Incubation period (days)									
		0		4		21		35		48	
Column fraction	Nitrogen species	No worms	Worms	No worms	Worms	No worms	Worms	No worms	Worms	No worms	Worms
Water	NH_4-N	0	0	0.0103 ±0.0019	0.0168 ±0.0038	0.0147 ±0.0124	0.0113 ±0.0153	0	0	0	0
	NO_3-N	4.0864	4.0864	3.0280 ±0.2543	2.7464 ±0.0713	1.2601 ±0.1185	0.4984 ±0.1033	0.7885 ±0.0663	0.2547 ±0.1275	0.1341 ±0.0286	0.0283 ±0.0258
	Organic-N	0	0	0.0118 ±0.0005	0.0126 ±0.0044	0.0311 ±0.0129	0.0183 ±0.0089	0.0060 ±0.0017	0.0029 ±0.0003	0.0137 ±0.0058	0.0026 ±0.0002
Sediment	NH_4-N	0	0	0.0227 ±0.0042	0.0433 ±0.0061	0.0474 ±0.0052	0.0423 ±0.0040	0.0330 ±0.0014	0.0350 ±0.0014	0	0.0186 ±0.0008
	NO_3-N	0	0	0	0	0	0	0	0	0	0
	Organic-N (soluble)	0	0	0.0048 ±0.0008	0.0056 ±0.0011	0.0038 ±0.0016	0.0029 ±0.0001	0.0048 ±0.0004	0.0042 ±0.0009	0.0036 ±0.0006	0.0020 ±0.0006
	Organic-N	0	0	0	0	0	0	0	0	0	0
Total		4.0864	4.0864	3.0775 ±0.2427	2.8247 ±0.0735	1.3571 ±0.0895	0.5731 ±0.1161	0.8323 ±0.0654	0.2965 ±0.1273	0.1514 ±0.0271	0.0515 ±0.0308

TABLE 3. Cumulative carbon loss (mg) from Canagagigue sediment overlain with water or nitrate-N in the presence and absence of oligochaete worms and incubated at 22°C for 13 d. Values are expressed as mean ± SD.

Overlying solution	Treatment	Time (days) 3	6	10	13
Water	No worms	4.00 ±0.35	8.74 ±0.68	18.56 ±1.08	27.86 ±2.54
	Worms	5.72 ±0.25	14.32 ±1.13	25.62 ±3.13	33.82 ±4.07
Nitrate-N	No worms	2.30 ±0.69	7.40 ±0.75	17.60 ±0.75	26.40 ±1.97
	Worms	6.08 ±0.37	14.46 ±0.63	29.76 ±0.94	40.80 ±0.99

whether or not the tubificid worms had any influence on the respiration rate of the sediment with and without nitrate-N in the overlying solution. Triplicate sediment columns, overlain with nitrate-N solution (10 mg/L) or distilled water, and with 40 worms each, were incubated at room temperature (22°C) for 13 days. CO_2 evolution was monitored and the cumulative loss of carbon from the columns was calculated (Table 3). At the end of the experiment there was no difference in the amount of carbon lost from control sediment when overlain with either nitrate-N solution or water. However, the presence of the worms increased the carbon loss by about 21% and the worms and nitrate-N treatment increased the carbon loss by nearly 58%. Thus the effect of the worms in increasing the carbon loss from the sediment is further accentuated by the presence of nitrate-N.

In the third experiment, the hypothesis that the worms might increase denitrification by increasing the movement of nitrate into the sediment was tested. Nitrate and chloride ions are known to behave similarly relative to their mobilities in natural systems. Since chloride undergoes little change in soil or sediment, this ion was used as an indicator of the rate of nitrate transfer into the sediment. ANOVA tests of the data obtained in this experiment (Fig. 4) showed that the loss of chloride from the overlying water of columns containing worms was significantly greater ($P < 0.01$) than that of the controls. It was concluded that the worms did accelerate the movement of chloride into the sediment and it is thus expected that movement of nitrate will similarly be influenced.

The effect of the worms on the redox profile of the sediment was also studied in one experiment. Readings taken at intervals over a 4-wk period showed no significant shift in Eh relative to depth or time in the presence or absence of worms. The Eh in the solution overlying the sediment averaged a constant +450 mV while at 1.0 cm below the sediment–water interface the mean Eh value was −250 mV. This sharp drop in the redox potential indicated that the oxidized layer was limited to less than 1.0 cm of the sediment.

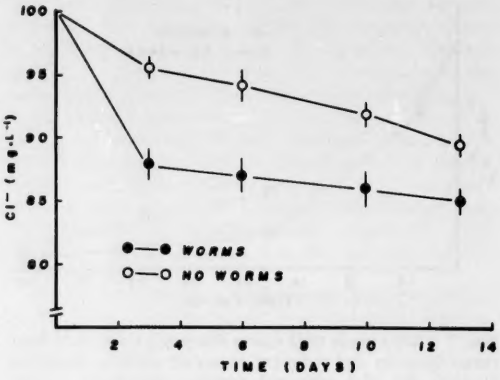

FIG. 4. Changes in chloride concentrations in a chloride solution overlying stream sediment incubated at 22°C for 13 d with and without oligochaete worms. Values are expressed as mean ± SD.

While the results to this point suggested some mechanisms by which the worms might enhance denitrification in the sediment, there remained the possibility that denitrification was also directly associated with the worms themselves. An experiment using micro-glass beads instead of sediment in the presence and absence of worms was conducted. Nitrate-N in the overlying liquid was monitored daily for 4 days. Then the worms were removed from the nitrate-containing columns with a Pasteur pipette, and transferred to the nitrate-containing columns so far devoid of worms. Some fecal pellets which sank into the glass-bead column were suspended at this time by stirring the top 2 cm of the beads with a glass rod to bring them into the aerobic zones of the columns. Nitrate-N was monitored for 3 more d. No change in nitrate-N concentration in the columns with distilled water was detected. In the remaining six tubes with nitrate as the overlying solution, a decrease in nitrate-N concentration seemed to be associated with the presence of worms (Fig. 5).

To find out whether the denitrifiers were located in the gut or on the outside surface of the worms, the microflora from these areas were tested for their denitrification capabilities. Forty worms (referred to as "starved") from the tubes with glass beads and nitrate used in the above experiment, and forty worms (referred to as "fed") freshly isolated from sediment provided the microbial populations. The MPN for denitrifiers outside and (presumably) inside the "starved" worms were 2.1×10^4 and 3.2×10^4, respectively (Table 4). The corresponding MPN in the case of the "fed" worms was lower than those of the "starved" worms. It is clear that nitrate-respiring organisms were present both within and outside the worms. The most common genera of microorganisms found

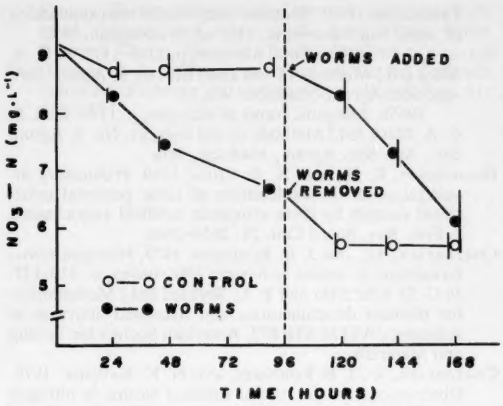

FIG. 5. Changes in the nitrate-N concentration in the nitrate solution overlying 4 cm of glass beads in tubes containing 40 or no oligochaete worms. The worms were transferred to the control (without worms) tubes after 96 h. Values are expressed as mean ± SD.

to be associated with the worms were *Pseudomonas* and *Flavobacterium*.

Discussion

If one accepts the premise that ^{15}N which was unaccounted for in the analyses conducted must have been denitrified, then the present study unequivocally proved that the tubificid worms significantly increased the rates of denitrification and nitrification in the stream sediment–water system. These experiments also confirmed earlier observations that the two processes occurred simultaneously (Chatarpaul et al. 1979) and that nitrate-N concentrations in the solution overlying sediment (Fig. 1, Tables 1 and 2) reflect the net result of nitrification and denitrification. It is therefore probable that published rates of denitrification based on the disappearance of unlabeled nitrate-N from water overlying stream sediments (Kaushik et al. 1975; Toms et al. 1975; Andersen 1976) were underestimates.

TABLE 4. Most probable numbers of denitrifiers and nitrate reducers found on the outside and in the gut (presumably) of 40 oligochaete worms. "Starved" worms were kept in 4 cm of glass beads overlain with nitrate-N solution for 7 d. "Fed" worms refer to those freshly isolated from sediment cultures.

Treatment	Location of isolate	Type of organisms	
		Denitrifiers	Nitrate reducers
"Starved" worms	Outside	2.1×10^4	17.0×10^4
	Guts	3.2×10^4	6.8×10^4
"Fed" worms	Outside	1.1×10^4	0.68×10^4
	Guts	0.46×10^4	0.40×10^4

The use of labeled nitrate-N made it possible to follow the sequence of nitrification–denitrification reactions. Nitrate-N added to the overlying solution diffuses down to the anaerobic layer where it is denitrified. Redox probes indicated that despite constant aeration of the water, this layer was just below the sediment–water interface. Meanwhile, ammonium-N originally present in the sediment plus that produced by mineralization diffuses to the aerobic surface layer where it is nitrified. The nitrate-N thus formed mixes with that present in the overlying water (as indicated by the dilution of ^{15}N isotope) and then diffuses into the anaerobic layer where it undergoes denitrification. A similar mechanism for sequential nitrification and denitrification in flooded soils was described by Patrick et al. (1976). Immobilization of nitrate-N appears to be insignificant because only trace amounts of the applied labeled N went into ammonium- and organic-N pools (Table 2).

Experiments with chloride solution (Fig. 4) supported the theory that the worms were able to enhance denitrification by increasing the movement of nitrate-N to sites for denitrification. This may be accomplished in the following manner. The worms are known to form numerous tunnels in the sediment as they search for food. Mechanical transfer of nitrate-N to the sediment may therefore be increased by the worms as they draw currents of nitrate-rich water into these tunnels. The tunnels then provide additional surface area for the diffusion of nitrate-N to actual denitrification sites. Edwards (1958) reported that on addition of *Chironomus riparius* larvae to settled activated sludge, there was an increase in ammonium concentration and a decrease in nitrate-N concentration in the overlying water. He also reported an increase in the depth of the oxidized zone of the sediment in the presence of the larvae. Andersen (1976) made similar observations when *C. plumosus* larvae were added to lake sediment.

The increase in nitrification (Fig. 3) may have resulted from the increased rate of sediment–water exchange of ammonium-N. The burrowing activities of the tubificids could quite likely accelerate the upward movement of sediment ammonium-N followed by oxidation in the aerobic layer. Ammonia excretion by the worms and subsequent oxidation to nitrate can also contribute to increased nitrate production in the columns with tubificids.

The observed increase in respiratory activity in the presence of nitrate-N (Table 3) could be accounted for by microbial denitrification. Andersen (1976) reported similar observations in the presence of *C. plumosus* larvae in lake sediment.

Results indicated that denitrifying bacteria were associated with the oligochaete worms and this is perhaps another reason for the greater rate of denitrification in their presence. The tubificids are known to graze on selected microbial species and only certain organisms seem to survive passage through their gut (Brinkhurst

and Chua 1969; Wavre and Brinkhurst 1971). Included in the latter group are species of *Pseudomonas*, *Bacillus*, *Micrococcus*, and *Flavobacterium*. All these genera are known to contain denitrifying forms. At least two of these, *Pseudomonas* and *Flavobacterium*, were identified in the present investigation. We cannot explain the larger most probable number of organisms associated with the "starved" than with the "fed" worms.

It is not clear exactly where on the worms denitrification was occurring since denitrifiers were isolated from the interiors as well as from the outside surface of the worms. Additional studies will be required to resolve these questions. However, it is tempting to speculate that the denitrification observed in the presence of the worms in the glass bead columns occurred in the worm gut, the nitrate-containing water having been drawn into the gut through the anus by the mechanism mentioned by Pennak (1953).

Except for our earlier paper (Chatarpaul et al. 1979), we are not aware of any study on the role of oligochaete worms in nitrogen transformations in stream sediments. While the present study was in progress, Kikuchi and Kurihara (1977) reported on the effects of the tubificids, *L. socialis* and *Branchiura sowerbyi*, on biological, chemical, and physical characteristics of submerged rice-field soil. They observed a rapid rise in oxidized nitrogen concentration after 45 d of incubation in the presence of the animals and concluded that the worms inhibited nitrification in the earlier stages of incubation. We now conclude, from our ^{15}N experiments, that the lower levels of nitrate in the initial stages resulted from rapid denitrification. Thus the importance of ^{15}N in similar studies cannot be overemphasized. Without its use in aquatic systems it is almost impossible to measure real nitrification because of concurrent denitrification.

In our investigation, it was demonstrated through the use of ^{15}N that denitrification and nitrification occurred simultaneously and were enhanced significantly in the presence of tubificid worms, *T. tubifex* and *L. hoffmeisteri*.

Acknowledgments

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ANDERSEN, J. M. 1976. Importance of the denitrification process for the rate of degradation of organic matter in lake sediments, p. 357–362. *In* H. L. Golterman [ed.] Interactions between sediments and freshwater. Junk, Amsterdam.

AMERICAN PUBLIC HEALTH ASSOCIATION, AMERICAN WATER WORKS ASSOCIATION, AND WATER POLLUTION CONTROL FEDERATION. 1971. Standard methods for the examination of water and wastewater. 13th ed. Washington, D.C.

BREMNER, J. M. 1965a. Total nitrogen, p. 1149–1178. *In* C. A. Black [ed.] Methods of soil analysis, No. 9. Agron. Ser., Am. Soc. Agron., Madison, Wis.

1965b. Inorganic forms of nitrogen, p. 1179–1238. *In* C. A. Black [ed.] Methods of soil analysis, No. 9. Agron. Ser., Am. Soc. Agron., Madison, Wis.

BRINKHURST, R. O., AND K. E. CHUA. 1969. Preliminary investigation of the exploitation of some potential nutritional sources by three sympatric tubificid oligochaetes. J. Fish. Res. Board Can. 26: 2659–2668.

CHATARPAUL, L., AND J. B. ROBINSON. 1979. Nitrogen transformations in stream sediment: ^{15}N studies, p. 119–127. *In* C. D. Litchfield and P. L. Seyfried [ed.] Methodology for biomass determinations and microbial activities in sediments, ASTM STP 673, American Society for Testing and Materials.

CHATARPAUL, L., J. B. ROBINSON, AND N. K. KAUSHIK. 1979. Observations on the role of tubificid worms in nitrogen transformations in stream sediment. J. Fish. Res. Board Can. 36: 673–678.

EDWARDS, R. W. 1958. The effect of larvae of *Chironomus riparium* Meigen on the redox potentials of settled activated sludge. Ann. Appl. Biol. 46: 457–464.

FIEDLER, R., AND G. PROKSCH. 1975. The determination of nitrogen-15 by emission and mass spectrometry in biochemical analysis: a review. Anal. Chim. Acta 78: 1–62.

FOCHT, D. D., AND H. JOSEPH. 1973. An improved method for the enumeration of denitrifying bacteria. Soil Sci. Soc. Am. Proc. 37: 698–699.

HILL, A. R. 1979. Denitrification in the nitrogen budget of a river ecosystem. Nature 281: 291–292.

KAUSHIK, N. K., AND J. B. ROBINSON. 1976. Preliminary observations on nitrogen transport during Summer in a small spring-fed Ontario stream. Hydrobiologia 49: 59–63.

KAUSHIK, N. K., J. B. ROBINSON, P. SAIN, H. R. WHITELEY, AND W. N. STAMMERS. 1975. A quantitative study of nitrogen loss from water of a small spring-fed stream, p. 110–117. *In* Water Pollut. Res. in Canada. Proc. 10th Canadian Symp., Toronto, Ont.

KIKUCHI, E., AND Y. KURIHARA. 1977. *In vitro* studies of the effects of tubificids on biological, chemical and physical characteristics of submerged rice-field soil and overlying water. Oikos 29: 348–356.

PATRICK, W. H. JR., R. D. DELAUNE, R. M. ENGLER, AND S. GOTOH. 1976. Nitrate removal from water at the water–mud interface in wetlands. Tech. Rep. EPA-600/3-76-042.

PENNAK, R. W. 1953. Fresh-water invertebrates of the United States. The Ronald Press Co., New York.

ROBINSON, J. B., H. R. WHITELEY, W. N. STAMMERS, N. K. KAUSHIK, AND P. SAIN. 1979. The fate of nitrate in small streams and its management implications, p. 247–259. *In* R. C. Lohr *et al.* [ed.] Best management practices for agriculture and silviculture. Ann Arbor Science, Mich.

SAIN, P., AND F. E. BROADBENT. 1977. Decomposition of rice straw in soils as affected by some management factors. J. Environ. Qual. 6: 96–100.

SAIN, P., J. B. ROBINSON, W. N. STAMMERS, N. K. KAUSHIK, AND H. R. WHITELEY. 1977. A laboratory study on the role of stream sediment in nitrogen loss from water. J. Environ. Qual. 6: 274–278.

TOMS, I. P., M. J. MINDENHALL, AND M. M. I. HARMAN. 1975.

Factors affecting the removal of nitrate by sediments from rivers, lagoons and lakes. Tech. Rep. TR14. Water Research Centre, Stevenage, Herts., England.

VAN KESSEL, J. F. 1977a. Removal of nitrate from effluent following discharge on surface water. Water Res. 11: 533–537.

1977b. Factors affecting the denitrification rate in two water-sediment systems. Water Res. 11: 259–267.

WAVRE, M., AND R. O. BRINKHURST. 1971. Interactions between some tubificid oligochaetes and bacteria found in the sediments of Toronto Harbour, Ontario. J. Fish. Res. Board Can. 28: 335–341.

Distribution and Biological Availability of Reactive High Molecular Weight Phosphorus in Natural Waters in New Zealand

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WHITE, E., AND G. PAYNE. 1980. Distribution and biological availability of reactive high molecular weight phosphorus in natural waters in New Zealand. Can. J. Fish. Aquat. Sci. 37: 664–669.

Eutrophic lakes and many streams on the central volcanic plateau of the North Island, New Zealand, contain dissolved chemically reactive phosphorus which is not orthophosphate. Sephadex gel (G25-150) chromatograms reveal that one reactive component is of high molecular weight (MW > 5000) while a second component elutes in the same way as phosphate-phosphorus (PO_4-P). The reactive high molecular weight phosphorus (RHMW-P) in streams generally forms only a small proportion of the dissolved reactive phosphorus (DRP). In some eutrophic lakes the proportion of RHMW-P in the DRP is high (70–80%). Each lake may have a characteristic distribution of the two forms of reactive phosphorus. In summer where DRP concentrations are low ($<2\ mg \cdot m^{-3}$) in lake surface waters, PO_4-P seems to dominate over RHMW-P which is consistent with long $^{32}PO_4$ turnover times which are found in many of the central volcanic plateau lakes. Algal growth responses to phosphorus uptake were similar for PO_4-P and RHMW-P, but not all of the RHMW-P was taken up by *Chlorella* in bioassays. The proportion of the RHMW-P taken up by *Chlorella* was different for each lake examined, posing problems in relating DRP concentrations to algal growth responses in lakes.

Key words: reactive phosphorus, phosphate-phosphorus, algae, *Chlorella*, growth, bioassay, freshwater analysis, molybdenum blue method, Sephadex gel chromatography

WHITE, E., AND G. PAYNE. 1980. Distribution and biological availability of reactive high molecular weight phosphorus in natural waters in New Zealand. Can. J. Fish. Aquat. Sci. 37: 664–669.

Les lacs eutrophes et plusieurs cours d'eau du plateau volcanique central de l'île North, en Nouvelle-Zélande, contiennent du phosphore chimiquement réactif dissous qui n'est pas de l'orthophosphate. Des chromatogrammes sur gel Sephadex (G25-150) indiquent qu'un composant réactif a un poids moléculaire élevé (MW > 5000), alors que l'élution d'un second composant se fait de la même manière que celle de l'orthophosphate (PO_4-P). Le phosphore de poids moléculaire élevé réactif (RHMW-P) dans les cours d'eau ne constitue ordinairement qu'une faible proportion du phosphore réactif dissous (DRP). Dans certains lacs eutrophes, la proportion de RHMW-P dans le DRP est élevée (70–80%). Les deux formes de phosphore réactif peuvent être réparties de façon caractéristique dans chaque lac. Pendant un été où les concentrations de DRP sont faibles ($<2\ mg \cdot m^{-3}$) dans les eaux superficielles d'un lac, le PO_4-P semble dominer le RHMW-P, ce qui s'accorde avec les longues périodes de renouvellement du $^{32}PO_4$ observées dans plusieurs lacs du plateau volcanique central. La croissance algale en réponse à l'absorption du phosphore est la même pour le PO_4-P que pour le RHMW-P, mais au cours d'essais biologiques, le RHMW-P n'est pas complètement absorbé par *Chlorella*. La proportion de RHMW-P absorbé par *Chlorella* diffère dans chacun des lacs examinés, ce qui soulève des problèmes quand il s'agit d'établir la relation entre les concentrations de DRP et la croissance des algues dans les lacs.

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DOWNES and Paerl (1978) described a method which enabled the separation of two dissolved reactive phosphorus (DRP) fractions from unconcentrated natural waters using Sephadex gels. They maintained that hydrolysis of high molecular weight material containing phosphorus by acid in the analytical reagents was the likely reason for this material being determined as DRP. Their observations were limited to three eutrophic lakes and one stream. In this study the range of natural waters examined for reactive high molecular weight phosphorus (RHMW-P) was extended to include a selection of streams and lakes exhibiting varied trophic conditions.

Paerl and Downes (1978) related their findings to the hypothesis of Rigler (1966, 1968) that the molybdenum blue technique often seriously overestimates the concentrations of orthophosphate in lake

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TABLE 1. Progressive dilution of Lake Ngahewa and Lake Tutaeinanga waters to determine the minimum concentration ($mg \cdot m^{-3}$) of reactive phosphorus that can be detected following Sephadex gel chromatography. DRP, dissolved reactive phosphorus; RHMW-P, reactive high molecular weight phosphorus; PO_4-P, phosphate-phosphorus; tr, trace present; ND, not detected.

Lake water	Dilution	DRP Expected	DRP Observed		RHMW-P	PO_4-P	RHMW-P/DRP
Ngahewa	×1		32.7	32.7	12.5	20.2	0.38
	×5	6.5	7.2	7.2	2.7	4.5	0.37
	×10	3.3	3.3	3.3	1.3	2.0	0.40
	×20	1.6	1.6	1.6	0.8	0.8	0.50
	×50	0.7	0.7	0.5	0.3	0.3	0.43
	×100	0.3	0.3	0.3	tr	tr	
Tutaeinanga	×1		51.5		38.5	13.0	0.75
	×5	10.3	10.2		7.3	2.9	0.71
	×10	5.2	4.7		3.6	1.1	0.77
	×20	2.6	3.2		2.2	1.0	0.68
	×50	1.0	0.7		0.5	tr	
	×100	0.5	0.5		tr	ND	

waters. The RHMW-P isolated by Downes and Paerl could be responsible for the discrepancies between the concentrations of phosphate-phosphorus (PO_4-P) and DRP demonstrated indirectly by Rigler. However, Downes and Paerl were not able to demonstrate this with certainty as their fractionations were limited to waters with dissolved reactive phosphorus concentrations >10 mg $P \cdot m^{-3}$, while Rigler's conclusions were based upon studies of epilimnetic waters in late summer when DRP concentrations were usually <2 mg $P \cdot m^{-3}$. In this study minor modifications to the chromatographic procedure of Downes and Paerl allowed lower concentrations of DRP to be identified with certainty so that the relative distribution of PO_4-P and RHMW-P could be examined in samples from the upper part of the concentration range covered in Rigler's studies.

Paerl and Downes (1978) found that phosphorus-starved *Chlorella* used PO_4-P and RHMW-P differentially. Growth responses were quicker when phosphate was available for use, but with a little extra time the RHMW-P material was used too. All DRP was removed from solution by the algae in their experiments and the yield of algal material was claimed to be similar from both phosphorus forms. Our bioassay studies used methodology similar to that of Paerl and Downes (1978) but were more extensive.

Materials and Methods

The chromatographic technique for separating RHMW-P (MW >5000) from PO_4-P was achieved using Sephadex gel (G25-150) in a column 26 cm long and 2 cm in diameter. The procedure differed from that of Downes and Paerl (1978) only in that a peristaltic pump controlled the flow rate at 2 $mL \cdot min^{-1}$, and the sample for analysis was applied without stopping the flow in the column at any time. Analysis of DRP in the effluent from the column was carried out on a continuous flow basis at a rate of 1 $mL \cdot min^{-1}$ with Technicon Auto-Analyzer II equipment using a manifold and analytical system designed by Downes (1978). These minor modifications allowed precise prediction of the timing of absorption peaks on the chart output, and permitted identification of DRP traces at concentrations as low as 0.3 $mg \cdot m^{-3}$ above background levels (Table 1). Qualitative comparisons of the relative peak sizes of RHMW-P and PO_4-P were possible on any chromatogram where concentrations of either component were in excess of 0.3 $mg \cdot m^{-3}$. Reproducible relative distributions of the two phosphorus forms were possible at concentrations in excess of 2 $mg \cdot m^{-3}$. Quantitative returns of DRP after chromatography were obtained from all of the lake waters studied in detail, for DRP concentrations measured directly by an autoanalyzer compared closely with estimates obtained by summing the areas of absorption peaks of RHMW-P and PO_4-P multiplied by absorption coefficients derived for phosphate standards. As the main object of the chromatographic studies was to compare the distribution of phosphorus in the two molecular weight fractions, the respective concentrations were calculated from a DRP analysis proportioned by absorption peak areas. The areas of absorption peaks produced by standard phosphate solutions as low as 8 $mg \cdot m^{-3}$ were often within 1% of each other when run at the beginning and end of a working day.

The rates of turnover of phosphate in lake waters were examined using carrier-free $^{32}PO_4$ in the form of phosphoric acid. The turnover times were obtained as described by Rigler (1956). Measurement of radioactivity in Millipore (0.45-μm porosity) filtrates were made initially with a Philips PW4633 gas flow low-level planchette geiger counter, the liquid being evaporated on the planchette. In later experiments measurements were made using a Beckman LS100C liquid scintillation counter. Samples treated with formalin (1% v/v) were run in parallel to test for abiotic ^{32}P adsorption.

The biological availability of RHMW-P and PO_4-P was examined using *Chlorella* as a bioassay organism. The algae were starved of phosphorus by growing them on a nutrient medium (Gorham et al. 1964) without phosphorus. Predetermined numbers of cells were added to 100-mL aliquots of water which were abundantly supplied with plant nutri-

TABLE 2. The distribution of two forms of dissolved reactive phosphorus at 1 m above the sediments of some Central Volcanic Plateau lakes, North Island, New Zealand, in April 1978. DRP, dissolved reactive phosphorus (in $mg \cdot m^{-3}$); RHMW-P, reactive high molecular weight phosphorus; PO_4-P phosphate-phosphorus (both expressed as percentages of the DRP concentration).

Lake	Trophic status	DRP	RHMW-P	PO_4-P
Taupo	Oligotrophic	5.0	0	100
Rotoma	"	2.3	0	100
Okataina	"	2.1	0	100
Tikitapu	"	14.0	0	100
Tarawera	Mesotrophic	9.2	0	100
Okareka	"	2.3	0	100
Rotoiti	"	95.6	1	99
Rotorua	Eutrophic	1.9	0	100
Ngahewa	"	93.0	76	24
Okaro	"	413	25	75
Rotongaio	"	592	2	98
Tutaeinanga	"	416	72	28

ents other than phosphorus. The initial *Chlorella* densities were constant in each experiment but ranged from 90 to 120×10^3 cells $\cdot mL^{-1}$ between experiments. Phosphorus was provided as phosphate in some cultures. In others, natural lake water was used so that mixtures of RHMW-P and PO_4-P made up the DRP complement. Other cultures were given no phosphorus. Each treatment had three replicates.

The cultures were kept in 125-mL Erlenmeyer flasks, and were incubated in a cabinet illuminated by "cool white" fluorescent lights at about 1300 lx for 18 h each day. Culture temperatures were not controlled, but in each experiment all cultures were exposed to the same temperature regime which varied between 18 and 22°C.

The growth responses were assessed by measuring cell numbers with a model TA Coulter counter. The bioassay experiments lasted longer than those of Paerl and Downes (1978), 7–14 d as opposed to 4 d, being terminated when it seemed that little further increase in cell numbers could occur.

Results and Discussion

Downes and Paerl noted that the three lakes in which they found RHMW-P were eutrophic, with predominantly agricultural catchments. A more extensive survey of New Zealand central volcanic plateau lakes was made to see if the presence of RHMW-P was associated with trophic condition and agricultural activities. Table 2 shows the relative abundance and concentration of DRP, RHMW-P, and PO_4-P found near the bottom of 12 lakes in April 1978. It is apparent that the RHMW-P is largely restricted to eutrophic lakes.

The distribution and abundance of RHMW-P was examined in 23 streams, including that studied by Downes and Paerl (1978). This particular stream contained the highest concentration ($46 mg \cdot m^{-3}$) and the highest proportion (46%) of RHMW-P, of all the streams examined. Four other streams arising from pastoral agricultural catchments contained from 1 to 28% RHMW-P in their DRP. This range of proportions was not different from that in other streams, so a definite association with agricultural activities could not be seen. Water from six streams was devoid of RHMW-P and as five of these arise as substantial springs, it suggests that underground waters do not contain this material.

TABLE 3. The distribution of reactive high molecular weight phosphorus and phosphate-phosphorus in surface waters in late summer where dissolved reactive phosphorus concentrations were low. DRP, dissolved reactive phosphorus; RHMW-P, reactive high molecular weight phosphorus; PO_4-P, phosphate-phosphorus (all in $mg \cdot m^{-3}$).

Lake	Date	DRP	RHMW-P	PO_4-P
Okaro	Mar. 30, 78	1.2	0	1.2
	Mar. 30, 78	2.5	0	2.5
	May 18, 78	1.4	0.3	1.1
	May 29, 78	6.3	2.3	4.0
Rotorua	Jun. 1, 78	1.5	0.4	1.1
Taupo	Mar. 14, 78	0.3	0	0.3
	May 29, 78	0.7	0	0.7

The ability to separate and identify RHMW-P and PO_4-P in waters containing low concentrations permits further examination of the significance of the relative abundance of these reactive phosphorus forms to Rigler's hypothesis (1966, 1968). To accommodate the hypothesis the distribution of RHMW-P relative to PO_4-P would favor the high molecular weight component, only 1–10% of the DRP being present as PO_4-P. Sephadex gel chromatograms of surface waters in late summer did not show this preponderance of RHMW-P (Table 3). The reverse was true, PO_4-P making up 63–100% of the DRP. These findings do not provide direct support for Rigler's hypothesis. They do not necessarily contradict it either because turnover rates between PO_4-P and sestonic phosphorus are rarely rapid in surface waters of central volcanic plateau lakes even in late summer. In this respect the phosphorus dynamics of these lakes are different to the Canadian lakes studied by Rigler (1964), who almost invariably found turnover times of less than 10 min in summer. Turnover times of less than 10 min have been found only in open water of lakes Okaro and Rotomahana. On one occasion when Okaro water had a rapid turnover time (4 min), Sephadex gel fractionation showed that the PO_4-P component approximated $1.1 mg \cdot m^{-3}$ while the RHMW-P was smaller ($0.3 mg P \cdot m^{-3}$), the opposite of that which Rigler might have hypothesized. Direct confirmation of Rigler's hypothesis might be achieved by employing the techniques described here on waters from the Canadian lakes he studied.

Phosphorus-starved *Chlorella*, at the densities which were used to inoculate culture media, took up PO_4-P within a few hours under the experimental conditions, while studies using Lake Tutaeinanga water showed

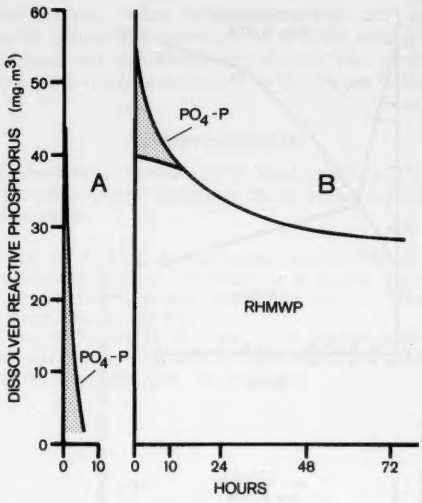

FIG. 1. Removal of phosphorus from solution with time by phosphorus-starved *Chlorella*. A, phosphorus supplied as phosphate (PO_4-P). B, filtered Lake Tutaeinanga water in which 25% of the reactive phosphorus is phosphate-phosphorus (PO_4-P) and the remainder is high molecular weight material (RHMW-P).

that the algae removed PO_4-P before much RHMW-P was taken up (Fig. 1). These observations were confirmed by phosphorus analyses of filtrates during bioassay experiments. Where PO_4-P was used as a nutrient, analyses at any time after 1 d revealed concentrations in solution of less than 1 mg·m^{-3} (all PO_4-P). Where natural lake waters were used, chromatograms showed that the DRP remaining in solution after the 1st d was almost all RHMW-P (Fig. 2). Knowledge of the rapid uptake of PO_4-P, and the slow uptake of RHMW-P in the presence of PO_4-P (Fig. 1), has been used to estimate the DRP concentration at day 1 for the bioassay studies (Fig. 2).

Several attempts were made to reproduce the *Chlorella* bioassay study described by Paerl and Downes (1978). They used water from Lake Tutaeinanga, in which the DRP was almost all in the form of RHMW-P (>98%). This condition was not found by us, PO_4-P making up 20–30% of the DRP in nine samples taken between April and July 1978. Samples were diluted to give concentrations of DRP of about 10 mg·m^{-3} and the growth response of phosphorus-starved *Chlorella* added to this water was compared with growth in PO_4-P solutions. We were unable to obtain satisfactory replication of results using such low starting concentrations of DRP. In particular the amount of phosphorus incorporated into new cells was very variable. Only one feature of the experiments was reproducible, 40–50% of the RHMW-P was not utilized by the *Chlorella*, a result very different from that of Paerl and Downes.

TABLE 4. The percentage frequency distribution of reactive high molecular weight phosphorus in dissolved reactive phosphorus found in water samples taken from three lakes. RHMW-P, reactive high molecular weight phosphorus.

	Percentage RHMW-P										
Lake	0	10	20	30	40	50	60	70	80	90	100
Tutaeinanga	0	0	0	0	0	0	0	9	0	1[a]	
Ngahewa	0	0	0	1	3	3	0	0	0	2[b]	
Okaro	3	3	6	2	0	0	0	0	0	0	

[a]Sample reported by Downes and Paerl (1978).
[b]Lake edge samples.

Two bioassays were carried out using both a selection of undiluted lake waters and phosphate standards at concentrations up to 50 mg·m^{-3} (Fig. 2). Cell numbers increased up to 15 times in these experiments before cell division ceased. The relationship between phosphorus uptake and cellular production was much less variable, average phosphorus content per cell being between 20 and 27 fg (1 femtogram ≡ 1×10^{-15} g) by the time cell division stopped. All experiments showed a reduction in phosphorus content per cell as cell numbers reached the asymptote. When algae were added to a medium containing 43 mg PO_4-P·m^{-3} (Fig. 1A), the cellular content reached 450 fg·cell^{-1} within 5 h, 20 times the content at the end of the bioassays. The uniform phosphorus content per cell at the end of the experiments offers support to the suggestion of Paerl and Downes (1978) that PO_4-P and the RHMW-P taken up can be utilized to the same extent in growth. Inspection of Fig. 2 suggests that in Lake Tutaeinanga, at least two forms of utilizable RHMW-P may exist, one which is taken up readily after PO_4-P is exhausted, and another which is taken up only slowly. In all experiments with mixtures of reactive phosphorus components, there seems to be an abrupt change of phosphorus uptake rate between 1 and 2 d, a slow rate replacing a faster rate, even though cell numbers increased rapidly after 2 d. As all of the natural lake waters contained PO_4-P, it was not possible to substantiate the view of Paerl and Downes (1978) that growth responses were quicker with PO_4-P than with RHMW-P. As with the bioassays using diluted lake water, all of the RHMW-P was not used. In Lake Tutaeinanga water 22–37% of it remained at the end of the bioassays. In Lake Ngahewa water 64–76% of the RHMW-P remained in solution, while in Lake Okaro water the figure was 27%. As the proportion of RHMW-P used by *Chlorella* varied from lake to lake, and ratios of PO_4-P to RHMW-P also varied between lakes (Table 4), the interpretation of DRP analyses in terms of biological availability is probably impossible wherever RHMW-P is present.

There was no evidence to suggest that RHMW-P was produced by *Chlorella* in the cultures. Those set up using PO_4-P solutions never showed any trace of RHMW-P when chromatographed during the course

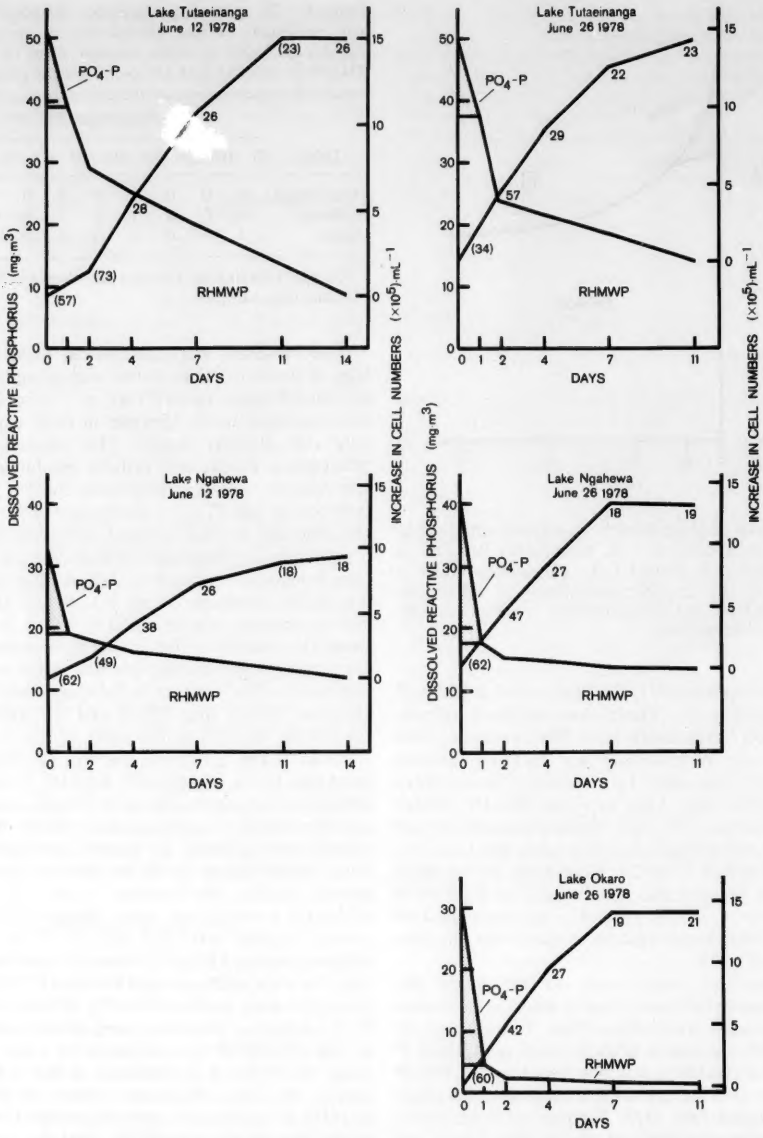

FIG. 2. Two experiments showing the removal of dissolved reactive phosphorus from Lakes Tutaeinanga, Ngahewa, and Okaro by phosphorus-starved *Chlorella*, and the resultant increase in algal cell numbers. Calculations of phosphorus content per cell (femtograms) are shown along the curve of cell numbers increase. Numbers in parentheses are calculated from the curves in the figures, not from counts of cell density and measurement of phosphorus uptake. PO_4-P, phosphate-phosphorus; RHMW-P, reactive high molecular weight phosphorus.

of the bioassay. When cultures were kept until algae decayed, increased concentrations of DRP were found in solution, but chromatographs showed that these increases were always attributable to PO_4-P, not RHMW-P.

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Downes, M. T. 1978. An automated determination of low reactive phosphorus concentrations in natural waters in the presence of arsenic, silicon and mercuric chloride. Water Res. 12: 743–745.

Downes, M. T., and H. W. Paerl. 1978. Separation of two dissolved reactive phosphorus fractions in lakewater. J. Fish. Res. Board Can. 35: 1636–1639.

Gorham, P. R., J. S. McLachlan, U. T. Hammer, and W. K. Kimm. 1964. Isolation and culture of toxic strains of *Anabaena flos aquae* (Lyngb.) de Breb. Int. Ver. Theor. Angew. Limnol. Verh. 15: 796–804.

Paerl, H. W., and M. T. Downes. 1978. Biological availability of low versus high molecular weight reactive phosphorus. J. Fish. Res. Board Can. 35: 1639–1643.

Rigler, F. H. 1956. A tracer study of the phosphorus cycle in lake water. Ecology 37: 550–562.

1964. The phosphorus fractions and the turnover time of inorganic phosphorus in different types of lakes. Limnol. Oceanogr. 9: 511–518.

1966. Radiobiological analysis of inorganic phosphorus in lakewater. Int. Ver. Theor. Angew. Limnol. Verh. 16: 465–470.

1968. Further observations inconsistent with the hypothesis that the molybdenum blue method measures orthophosphate in lake water. Limnol. Oceanogr. 13: 7–13.

Seasonal Factors Affecting Buoyancy Attained in Still Water and Current by Fathead Minnows, *Pimephales promelas*

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This study investigated effects of various seasonal factors on buoyancy attained by fathead minnows, *Pimephales promelas*. Fish were taken from the field to the laboratory at regular intervals (when possible) over 2 yr, and buoyancy attained in either still water or current was measured. Buoyancy attained in still water and in current (maximum tolerable velocity) varied seasonally when fish were held and tested at a natural photoperiod and temperature of capture. Seasonal variation still occurred when fish were held and tested at a natural photoperiod but at 15°C, and for those in current, at a velocity of 35 $cm \cdot s^{-1}$. There was no seasonal variation in buoyancy attained by fish held in the laboratory under constant environmental conditions. Effects of photoperiod, sex, gonad development, estimated fat content, and condition of the fish on buoyancy were determined. Fish exposed to a photoperiod of 12 h illumination were less buoyant in still water and current than those held under longer or shorter photoperiods. There was no significant difference in buoyancy attained between females and males. However, buoyancy decreased significantly, especially in current, as gonad development increased. Buoyancy increased significantly in still water and in current with days of starvation. As coefficient of condition decreased, buoyancy increased significantly in current but not in still water. Estimated fat content, determined by a densitometric method, had no significant effect on buoyancy. Seasonal variation occurs in the ability to attain a particular buoyancy and this is influenced by a number of potentially interacting factors that include water temperature and velocity, photoperiod, gonad development, age, and condition of the fish.

Key words: fathead minnow, *Pimephales promelas*, buoyancy, seasonal variation, photoperiod, condition of fish, gonad development

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Notre étude porte sur les effets de divers facteurs saisonniers sur la flottabilité réalisée par le tête-de-boule, *Pimephales promelas*. Les poissons furent capturés sur le terrain et transportés au laboratoire à intervalles réguliers (lorsque possible) sur une période de 2 ans, et nous avons mesuré la flottabilité atteinte, soit en eau morte soit en eau courante. La flottabilité atteinte en eau morte et dans un courant (vitesse tolérable maximale) varie selon la saison quand les poissons sont maintenus et traités à une photopériode naturelle et à la température à laquelle ils ont été capturés. Il y a encore variation saisonnière quand les poissons sont maintenus et traités à une photopériode naturelle, mais à une température de 15°C et, dans le cas de poissons maintenus dans un courant, à une vitesse de 35 $cm \cdot s^{-1}$. On n'observe pas de variation saisonnière de flottabilité atteinte par les poissons maintenus en laboratoire dans des conditions ambiantes uniformes. Nous avons déterminé les effets de la photopériode, sexe, développement des gonades, teneur estimée en gras et condition du poisson sur la flottabilité. Les poissons exposés à une photopériode de 12 h de clarté ont une flottabilité moindre en eau dormante et courante que ceux maintenus à des photopériodes plus longues ou plus courtes. Il n'y a pas de différence significative de flottabilité atteinte par femelles et mâles. Cependant, la flottabilité diminue de manière significative, surtout dans un courant, à mesure que les gonades se développent. La flottabilité augmente de manière significative en eau morte et courante en fonction du nombre des jours de jeûne. A mesure que diminue le coefficient de condition, la flottabilité augmente nettement en eau courante, mais non en eau dormante. La teneur estimée en gras, déterminée par méthode densimétrique, n'a pas d'effet important sur la flottabilité. Il y a variation saisonnière d'aptitude à atteindre une flottabilité particulière, et cette aptitude est influencée par plusieurs facteurs à interactions possibles, tels que température et vitesse de l'eau, photopériode, développement des gonades, âge et condition du poisson.

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THE ability to reduce swim bladder volume and thus buoyancy as water velocity increases, and to increase swim bladder volume to attain near neutral buoyancy in still water is a common adaptation among North American stream-dwelling fish (Gee et al. 1974). The appropriate buoyancy permits efficient movement in still water and maintenance of position in faster waters with minimum expenditure of energy (Saunders 1965; Gee et al. 1974; Gee and Gee 1976; Berezay and Gee 1978).

Buoyancy is affected by the size of fish (Gee 1968, 1972, 1977; Machniak and Gee 1975; Berezay and Gee 1978), water velocity (Neave et al. 1966; Gee 1977), water temperature (Pinder and Eales 1969; Gee 1977), and by interactions between these factors (Berezay and Gee 1978). Other factors affecting buoyancy, especially those related to season, remain largely unknown. Neave et al. (1966) and Pinder and Eales (1969) found seasonal variations in buoyancy of juvenile Atlantic salmon (*Salmo salar*) in still water and in current. These variations were related to size of fish and water temperature.

The greatest demand on temperate stream fish to hold position is during spring runoff when velocities are greatest and waters are cold. Yet, the extent of reduction in buoyancy in current by fathead minnows (*Pimephales promelas*) is minimal at low temperatures (Gee 1977). Gee (1977) suggested that other variables related to seasonal change, such as photoperiod or increasing water temperature, could stimulate a greater decrease in buoyancy at low temperature ranges. The purpose of this study was to assess effects of season and its dependent variables on maximum and minimum buoyancy attained by fathead minnows.

Materials and Methods

Fathead minnows were collected from Crystal Creek, Manitoba, and transported to the laboratory in styrofoam coolers with Plexiglas windows in the top, thus maintaining field photoperiod and water temperature. Fish were fed Tetramin flakes or Trout Starter (No. 3) once a day, except 24 h prior to buoyancy measurements.

To determine maximum and minimum buoyancy, fish were held in either still water or current, respectively, for ~24 h (Gee 1977). Current was created in an aquarium (90 × 44 × 44 cm) using the design of Gee and Bartnik (1969). Water depth was about 6 cm and maximum water velocity was set such that all fish could hold position without resting against the back screen of the stream tank. The mean velocity in any vertical velocity curve occurs at about six-tenths of the depth (Grover and Harrington 1966). Therefore, water velocity was recorded by averaging six measurements from different locations taken 2.5 cm from the bottom with an Ott current meter (type C1). Still water conditions were created in an aquarium (90 × 44 × 44 cm) using only a gently bubbling airstone. Water (dechlorinated by the charcoal method) was continuously exchanged in all aquaria to prevent buildup of wastes.

To measure buoyancy, fish were dip-netted, anesthetized with MS-222 (ethyl *m*-aminobenzoate methanesulfonate), and the estimated swim bladder volume and weight of gas-free fish in water were recorded using the procedure of Gee (1970). The temperature of the anesthetic solution and the water in the holding and experimental tanks were similar. Buoyancy was expressed by dividing the estimated swim bladder volume (±0.001 mL) by the weight (±0.001 g) of the gas-free fish in water, where 1.0 $mL \cdot g^{-1}$ is neutral buoyancy. The difference between the weight (g) of the fish in water with its swim bladder inflated and deflated was used as an estimate of swim bladder volume. No correction to swim bladder volume was made for depth of capture (Gee et al. 1974). All buoyancy measurements were made during midday.

SEASONAL FACTORS

To determine if maximum (in still water) and minimum (in current) buoyancy varies (A) seasonally with water velocity and temperature, photoperiod, and other related factors and (B) seasonally but independent of variation in water velocity and temperature, fish were collected from the field, divided into two groups, and each group held and tested under the appropriate conditions (Table 1). Fish

TABLE 1. Plan of experiment to determine if maximum and minimum buoyancy varies (A) seasonally, (B) seasonally but independent of variation in water velocity and temperature, and (C) seasonally but independent of variation in direct environmental cues.

Group	A	B	C
Source of fish	Collected from Crystal Creek, once a month when possible from June 1976 to May 1978		Collected from Crystal Creek in May 1976
Laboratory holding conditions	Held at field photoperiod and water temperature for 6 d prior to testing	Held at field photoperiod and acclimated to 15°C for 7 d prior to testing	Held at a photoperiod of 12 h and 5°C. Fish were acclimated to 15°C for 8 d prior to testing
Testing conditions	Tested at field photoperiod and water temperature. One batch in still water and one in maximum water velocity tolerated by all fish (20–53 $cm \cdot s^{-1}$) for 24 h	Tested at field photoperiod and 15°C. One batch in still water and one in a velocity of 35 (±2) $cm \cdot s^{-1}$ for 24 h	Tested at a photoperiod of 12 h and 15°C. One batch in still water and one in a velocity of 35 (±2) $cm \cdot s^{-1}$ for 24 h
Duration of experiment (months)	24	24	16

were collected approximately once a month from June 1976 to May 1978 when possible. Group A fish were held in the laboratory for 6 d before testing buoyancy, as this was the minimum time found for fish to recover from the stress of capture and transportation (Luoma 1979). The lowest water temperature that could be obtained in the laboratory was 5°C. Therefore, fish collected from temperatures less than this were held and tested at 5°C. Fish were acclimated to desired temperatures by allowing at least 1 d for every 1.5°C change. Temperatures were controlled to within ±0.5°C. Photoperiod (hours of daily illumination) was regulated by 60-W incandescent bulbs on 24-h time clocks. To determine if maximum and minimum buoyancy varies (C) seasonally but independent of variation in direct environmental cues, fish were collected in May 1976 and acclimated to laboratory conditions for 2 mo before the start of this experiment (Table 1). Group C fish were held at 5°C to retard growth and then acclimated to 15°C for 8 d before testing buoyancy to compare results with group B. Batches of fish from group C were tested at times of the year similar to those of groups A and B. In each group, buoyancy measurements were made on eight fish from still water and eight fish from current. Fish tested were of similar size (4.3–5.7 cm, fork length) and sexes were used at random.

In addition to buoyancy measurements, sex and the coefficient of condition (K) were determined. K was calculated according to the method of Hile (1936), where K = weight in air (g) ÷ length^3 (cm) × 100.

Photoperiod, sex, gonad development, estimated fat content, and condition of the fish were then further studied to determine their influence on seasonal variations in buoyancy found in groups A and B. These factors were tested on fish collected in October and November 1977 that were held prior to testing at 11°C and at a photoperiod of 12 h.

Photoperiod

To determine effects of exposure to various photoperiods on maximum and minimum buoyancy, groups of fish (4.5–5.7 cm) were acclimated in December 1977 to 15°C and photoperiods of either 9, 10.5, 12, 13.5, 15, or 16.5 h for 3 wk. At each photoperiod eight fish were held in either still water or current (35 ± 2 cm·s^{-1}) at 15°C for 24 h before measuring buoyancy.

Sex and Gonad Development

To determine effects of sex and gonad development on maximum and minimum buoyancy, adult fish (5.0–7.0 cm) were held in four aquaria (90 × 44 × 44 cm) at a density of about 45 fish per tank and gradually acclimated to 21°C and to a photoperiod of 16 h over 5 wk. Each aquarium contained a gently bubbling airstone and an opaque cover to decrease light intensity from a 40-W overhead light. Spawning sites consisted of PVC piping and broken clay flower pots. Approximately once a month for 4 mo, from March to June 1978, batches consisting of about 10 female and 10 male fish were held in still water and current (40 ± 2 cm·s^{-1}) at 21°C for 24 h before measuring buoyancy. Fish were drip-dried for 1 h to obtain total body weight and gonads were then removed and weighed (±0.001 g). The weight of the gonads divided by the total body weight was used as an index of gonad development. The largest gonad:body weight ratio for each sex was assumed to be 100% gonad development. Gonad development was then calculated for each sex by the formula:

$$\%\ \text{gonad development} = \frac{\text{gonad:body weight ratio}}{\text{largest gonad:body weight ratio}} \times 100.$$

Condition and Estimated Fat Content

The effect of condition on maximum and minimum buoyancy was examined in fish (5.2–6.7 cm) acclimated to 15°C and a photoperiod of 12 h for 4 wk. On day 0, normal daily feeding ceased and all debris was removed from the aquarium. Buoyancy was measured after 0, 4, 8, 12, 16, 20, 24, 36, and 60 d of starvation during May and June 1978. Two batches of eight fish were taken at the above times and one was held in still water and the other in current (35 ± 2 cm·s^{-1}) at 15°C for 24 h before measuring buoyancy. The coefficient of condition (K), used to describe the degree of starvation, was determined and related to variation in buoyancy.

The effect of estimated fat content on maximum and minimum buoyancy was determined in these starved fish, using the indirect method of Horak (1966) to estimate fat content:

$$\%\ \text{fat} = 100 \times \left[\frac{\text{Df}}{\text{Dff} - \text{Df}}\right] \times \left[\frac{\text{Dff}}{\text{specific gravity}} - 1\right]$$

where Df is the density of body fat (0.9348), Dff is the density of the fat-free body (1.1000), and specific gravity is determined by $\frac{\text{Wa} \times K}{\text{Wa} - \text{Ww}}$ where Wa is the body weight of the fish in air, Ww is the weight in water, and K is the density of water containing the fish at 15°C (0.99913, from a density table).

Statistical Analysis

Statistical analysis of data was done on an IBM/370 computer using APL statistical library program 5796-PHW. Data were analyzed using one- and two-way analysis of variance, analysis of covariance, single linear regression analysis, and Bartlett's test (homogeneity of variances). All differences were considered to be significant when $P < 0.05$.

Results

Seasonal Factors

Group A fish — Both maximum (in still water) and minimum (in current) buoyancy varied significantly with time of year (Fig. 1A). The pattern of variation also differed between the 2 yr of the study. Fish were close to neutral buoyancy in still water, varying from a mean of 0.900 to 1.015 mL·g^{-1}, with the lowest buoyancy values occurring in breeding fish and the highest values in the coldest waters. In current, fish were negatively buoyant, varying from a mean of 0.523 to 0.877 mL·g^{-1}, with the lowest buoyancy values in May or June of each year. This coincides with the potential for high tolerable water velocity and high water temperature, a long photoperiod, and the start of the breeding season. Fish tested in April of each year during spring runoff showed a minimal ability to reduce buoyancy in current, especially in 1977 (Fig. 1A), when fish were parasitized by metacercaria of a digenetic trematode.

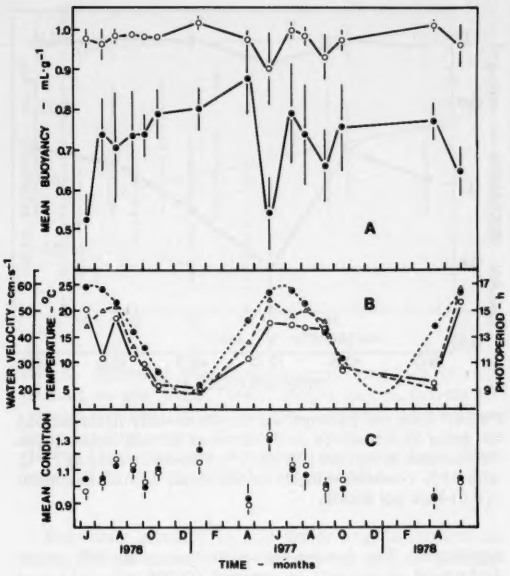

FIG. 1. (A) Mean buoyancy ($n = 8$) attained by group A fish over 2 yr of study in still water (open circles) and in current (closed circles). Vertical lines represent 95% confidence limits on the mean (those <0.014 are not shown). (B) Maximum tolerable water velocity (○), water temperature (△), and photoperiod (●) at which fish were tested. (C) Mean coefficient of condition ($n = 8$) for fish tested in still water (open circles) and in current (closed circles). Only one side of the 95% confidence limits on the mean is shown (those <0.030 are not shown).

The maximum tolerable water velocity, water temperature, and photoperiod at which fish were tested varied throughout the year (Fig. 1B). The coefficient of condition varied seasonally with the lowest values occurring in April and the highest value in May 1977 in breeding fish (Fig. 1C). Single linear regressions of buoyancy attained in still water on water temperature, photoperiod, and coefficient of condition were significant in group A fish. In current, regressions of buoyancy on water velocity, water temperature, photoperiod, and coefficient of condition were also significant. The slope of the regression line and the percentage of the total variation that is explained by the regression (r^2) for each factor are as follows:

	Still water		Current	
Factor	Slope	r^2(%)	Slope	r^2(%)
Water velocity	—	—	−0.0057	20.0
Water temperature	−0.0025	10.3	−0.0076	12.2
Photoperiod	−0.0041	4.2	−0.0187	10.9
Condition	−0.0787	4.5	−0.2470	4.2

Group B fish — Independent of variation in water velocity and temperature, both maximum and minimum

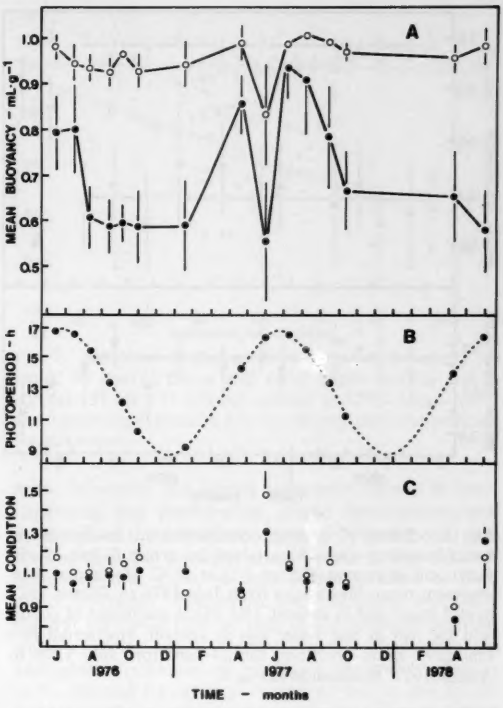

FIG. 2. (A) Mean buoyancy ($n = 8$) attained by group B fish over 2 yr of study in still water and in current (35 cm·s^{-1}) at 15°C. (B) Photoperiod at which fish were tested. (C) Mean coefficient of condition for fish in still water and in current. Notation as in Fig. 1.

buoyancy varied significantly with time of year (Fig. 2A). In still water, fish were close to neutral buoyancy varying from a mean of 0.833 to 1.006 mL·g^{-1} and in current buoyancy varied from a mean of 0.556 to 0.933 mL·g^{-1}. The ability to reduce buoyancy in current was minimal in midsummer of each year and when fish were parasitized in April 1977 (Fig. 2A). Photoperiod and coefficient of condition were the factors varying over time for group B fish (Fig. 2B and C). The pattern of variation also differed between groups A and B fish (Fig. 1A and 2A).

The single linear regression of buoyancy attained in still water on coefficient of condition was significant, but photoperiod was not significant in group B fish. In current, regressions of buoyancy on photoperiod and coefficient of condition were significant. The slope of the regression line and r^2 for each factor are as follows:

	Still water		Current	
Factor	Slope	r^2(%)	Slope	r^2(%)
Photoperiod	Not significant		0.0225	11.2
Condition	−0.1099	8.3	−0.3619	8.2

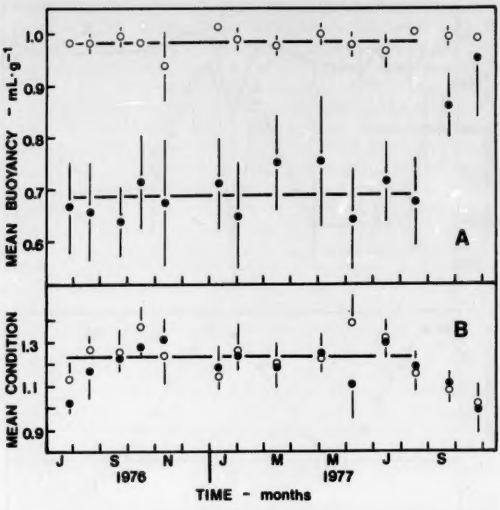

FIG. 3. Effect of constant environmental conditions on mean buoyancy ($n = 8$) attained by group C fish in still water and in current (35 cm·s^{-1}) at 15°C. Horizontal lines represent mean buoyancies from July 1976 to August 1977 in still water and in current. (B) Mean coefficient of condition for fish in still water and in current. Horizontal line represents mean condition for all fish from July 1976 to August 1977. Notation as in Fig. 1.

Group C fish — For fish held under constant laboratory conditions of a 12-h photoperiod and 15°C, the effect of time of year on maximum and minimum buoyancy was not significant over the first 14 mo (Fig. 3A). There was a significant difference between buoyancy in still water and in current. In still water, fish were close to neutral buoyancy with an overall mean of 0.981 mL·g^{-1} and in current the overall mean negative buoyancy was 0.686 mL·g^{-1} (Fig. 3A). Interaction between water velocity and time was not significant and thus extent of buoyancy adjustment was similar at all times over the first 14 mo. The extent of buoyancy adjustment is the difference in mean buoyancy between still water and current.

The coefficient of condition increased several months after the fish were brought into the laboratory and then remained relatively constant over the first 14 mo, with an overall mean of 1.229 (Fig. 3B). Fish tested in September and October 1977 showed a decrease in coefficient of condition, and mean buoyancy attained in current increased to almost neutral (Fig. 3A). Group C fish gradually died from September to November 1977.

PHOTOPERIOD

Effects of water velocity (still or current) and photoperiod on buoyancy attained by medium-sized fish were

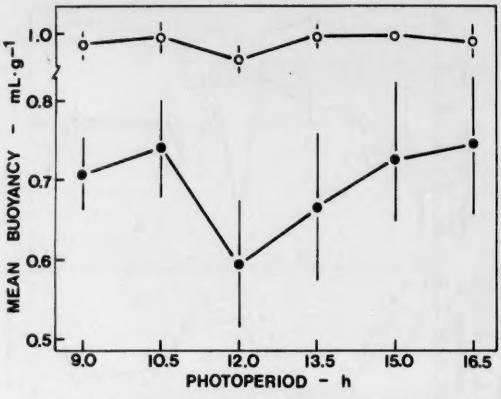

FIG. 4. Effect of photoperiod (hours of daily illumination) on mean buoyancy ($n = 8$) attained in still water (open circles) and in current (35 cm·s^{-1}) (closed circles) at 15°C, with 95% confidence limits on the mean. Confidence limits <0.014 are not shown.

significant. The lowest mean buoyancy in still water (0.968 mL·g^{-1}) and in current (0.596 mL·g^{-1}) was attained at a photoperiod of 12 h (Fig. 4). Interaction between the effects of water velocity and photoperiod was not significant and thus extent of buoyancy adjustment was similar at all photoperiods.

SEX AND GONAD DEVELOPMENT

Slopes of regression lines for female and male fish were not significantly different in either still water or current. There was also no significant difference between treatments (sex), in either still water or current. Therefore, single regression lines of $Y = 0.979 - 0.0007\,X$ for still water and $Y = 0.784 - 0.0042\,X$ for current suffice for both sexes (Fig. 5).

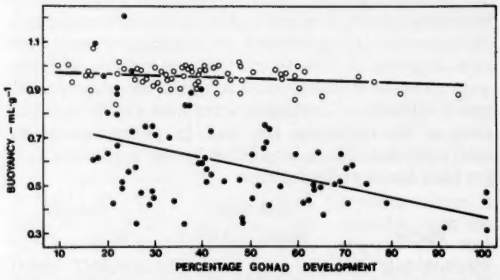

FIG. 5. Effect of gonad development on buoyancy attained in still water (open circles) and in current (40 cm·s^{-1}) (closed circles) at 21°C. Lines represent significant regression lines for combined female and male fish in still water and in current.

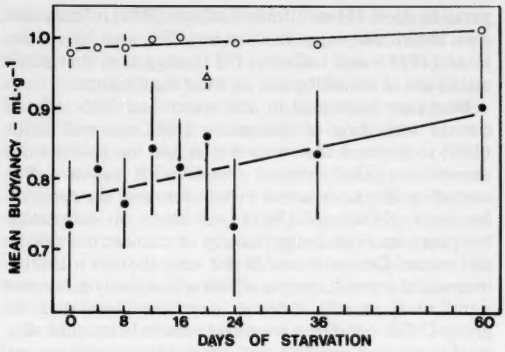

FIG. 6. Effect of starvation on mean buoyancy ($n = 8$) attained in still water (open circles) and in current (35 $cm \cdot s^{-1}$) (closed circles) at 15°C, with 95% confidence limits on the mean. Confidence limits <0.014 are not shown. Lines represent significant regression lines. Mean indicated by △ was omitted from analysis.

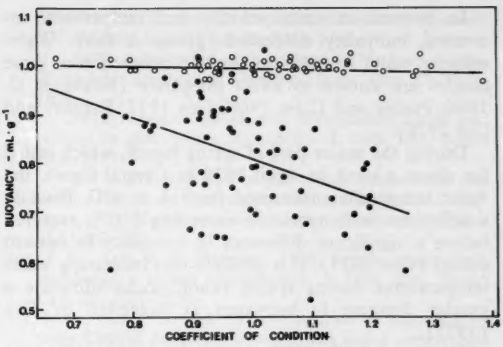

FIG. 7. Effect of coefficient of condition on buoyancy attained by starved fish in still water (open circles) and in current (35 $cm \cdot s^{-1}$) (closed circles) at 15°C. Lines represent significant regression line for current and nonsignificant for still water.

Buoyancy attained in still water and in current decreased significantly with increasing gonad development, with a greater decline occurring in current (Fig. 5). Thus extent of buoyancy adjustment increased with increasing gonad development.

CONDITION AND ESTIMATED FAT CONTENT

Mean buoyancy of starved fish increased significantly with increasing time of starvation in both still water and in current (Fig. 6). Regression lines were $Y = 0.985 + 0.0004\ X$ for still water and $Y = 0.771 + 0.0021\ X$ for current.

The relationship between coefficient of condition and buoyancy attained in still water was not significant, but buoyancy attained in current decreased significantly with increasing coefficient of condition (Fig. 7). The regression line was $Y = 1.240 - 0.4348\ X$. Thus extent of buoyancy adjustment was greatest among fish with the highest coefficient of condition.

During the starvation experiment, estimated fat content in fish decreased from 28.1 to 16.2% but there was a nonsignificant effect of estimated fat content on buoyancy attained in either still water or in current.

Discussion

Fathead minnows attained a near neutral buoyancy in still water and reduced buoyancy when exposed to current. There was seasonal variation in maximum and minimum buoyancy (group A fish) depending upon seasonal factors such as maximum tolerable water velocity, water temperature, photoperiod, and condition of the fish. The pattern of variation differed between the 2 yr of the study. Independent of variation in water velocity and temperature, maximum and minimum buoyancy still varied seasonally (group B fish), suggesting that photoperiod, gonad development, and condition of the fish affect buoyancy. The seasonal pattern of variation between group A and B fish differed, indicating that water velocity or temperature or both affect buoyancy. Maximum and minimum buoyancy did not vary with time of year under constant environmental conditions (group C fish) suggesting no endogenous influence on buoyancy. Buoyancy appears to be affected by external factors so that it can be adjusted rapidly to sudden and unexpected changes in the environment. This type of behavioral plasticity is common in North American stream fish (Gee et al. 1974).

WATER TEMPERATURE, VELOCITY, AND PHOTOPERIOD

In still water, as temperature decreased, buoyancy increased from slightly negative to positive (group A fish). Similar effects of temperature on fathead minnows were obtained by Gee (1977). Such a positive buoyancy at very low temperatures could be adaptive. Magnuson and Karlen (1970) showed that in an ice-covered lake oxygen depletion started at the bottom and proceeded upward in the water column. In late winter the highest concentration of dissolved O_2 was found at the ice–water interface. Fathead minnows were observed to move upward in the water column as oxygen became depleted at lower levels (Mills 1972). Thus, a positive buoyancy at low temperatures would allow fish to remain at the ice–water interface with minimum energy expenditure. Fathead minnows were also observed to irrigate their gills with water drawn over the surface of gas bubbles containing oxygen found at the ice–water interface or to take a bubble into their buccal cavity and pass water over it (Klinger 1978). During winter sampling fathead minnows were observed to be positively buoyant, remaining at the water surface in holes recently augered through the ice.

In current, as water velocity and temperature increased, buoyancy decreased (group A fish). Water velocity, water temperature, and an interaction of these factors are known to affect buoyancy (Neave et al. 1966; Pinder and Eales 1969; Gee 1977; Berezay and Gee 1978).

During the major part of spring runoff, which lasted for about a week in April 1978 in Crystal Creek, the water temperature increased from 1 to 4°C. Because a difference in temperature exceeding 5°C is required before a significant difference in buoyancy in current occurs (Gee 1977), it is unlikely that increasing water temperatures during spring runoff could stimulate a greater decrease in buoyancy as suggested by Gee (1977).

Photoperiod affected buoyancy attained in still water and in current with the lowest values occurring at a photoperiod of 12 h. This approximates late March and early April photoperiods when spring runoff usually occurs. Thus in terms of photoperiod, the potential for attaining the lowest buoyancy coincides with the time of greatest demand (spring runoff).

Size of fish and water temperature appear to interact with photoperiod to influence buoyancy further. In current (35 $cm \cdot s^{-1}$), small fathead minnows (3.3–4.5 cm) reached a minimum buoyancy at a photoperiod of 12.5 h whereas larger fish (5.5–6.8 cm) reached minimal values at 12.5 and 14.5 h (Luoma 1979). An analysis of the buoyancy of group A fish (held and tested at temperature of capture) showed that maximum (in still) and minimum (in current) buoyancy was negatively correlated with photoperiod while minimum buoyancy of group B fish (held and tested at 15°C) was positively correlated with photoperiod and maximum buoyancy remained unchanged. In nature a change in photoperiod is usually accompanied by a change in temperature that can strengthen or weaken the photoreactive response of various fishes (Girsa 1972). During spring runoff low water temperatures may interact with photoperiod to restrict the ability to lower buoyancy and ultimately hold position in faster water velocities.

Gonad Development, Condition, and Estimated Fat Content

There was no difference in maximum or minimum buoyancy attained between female and male fish. Buoyancy decreased as gonad development increased, with a greater decline occurring in current than in still water. Group A and B fish also attained the lowest buoyancy in still water and in current when in breeding condition.

The coefficient of condition is a numerical representation of robustness. Condition varied seasonally in group A and B fish, with the lowest values occurring in the spring and highest in breeding fish. Condition can be affected by sex, length, age, maturity, and environmental factors such as food supply, temperature, and parasitization (Hoar 1939; LeCren 1951). There are also individual variations among fish and therefore, Hoar (1939) and LeCren (1951) suggested that small variations in condition are of little significance.

Buoyancy increased in still water and especially in current with days of starvation. MacLeod and Smith (1966) observed that over 4 d active metabolism decreased in unfed fathead minnows. It appears that starvation decreases active metabolism and the capacity for work (MacLeod 1967). As condition decreased, buoyancy increased significantly in current but not in still water. Group A and B fish also showed a similar increase in current, and in addition buoyancy increased significantly in still water as condition decreased. In group C fish, condition remained relatively constant due to the constant feeding and laboratory conditions, as did maximum and minimum buoyancy. This suggests that the more robust a fish, the greater its ability is to reduce buoyancy in current.

Group C fish tested at the end of the experiment were 26 to 29 mo old and fathead minnows are rarely known to live beyond age 2 in nature (Scott and Crossman 1973). It appears that very old fish have a reduced ability to decrease buoyancy in current, either directly due to old age or indirectly due to the poor condition (low K values). Fish that were parasitized also showed a poor ability to reduce buoyancy in current. However, these fish (group A and B in April 1977) were also influenced by other seasonal factors, so that it is not certain whether parasitism affects buoyancy.

Estimated fat content had no significant effect on maximum or minimum buoyancy. The formula used to estimate fat content was initially developed for rainbow trout. Even though the values may not represent actual body fat concentrations in fathead minnows, differences in fat concentration would be apparent between individuals of the same population (Horak 1966). Variations in fat content have also shown little influence on density differences of fish tissues and therefore, swim bladder volume in various Cyprinidae (Taylor 1922; Alexander 1959, 1967) and in juvenile Atlantic salmon (Pinder and Eales 1969). However, maintenance of neutral buoyancy in some marine fish may be aided by the presence of large quantities of lipid in various body organs (Brawn 1969; Butler and Pearcy 1972; Bone 1973; Lee et al. 1975; DeVries and Eastman 1978).

Significance of Seasonal Factors Affecting Buoyancy

In April the ability of group A fish to reduce buoyancy in the laboratory was minimal and the maximum water velocity that they could withstand was 20 $cm \cdot s^{-1}$. Water velocities in Crystal Creek during spring runoff in April 1978 were as high as 48 $cm \cdot s^{-1}$. Although maximum reduction in buoyancy occurs during the April photoperiod, low water temperatures and a low coefficient of condition do not permit the potential

reduction in buoyancy to occur. Thus, to hold position under these conditions fathead minnows must seek nearby sheltered areas of reduced water velocity to avoid being displaced downstream. The latter can occur (Luoma 1979). Downstream displacement may not be a disadvantage. Winter conditions in streams of the great plains of North America often cause at least partial winter kill. Thus a reduced ability to lower buoyancy in current and subsequent drifting downstream may be a mechanism of dispersal repopulating suitable areas of a stream prior to reproduction. Fathead minnows are common in the headwaters of streams as well as in the lower reaches and hence a downstream displacement could be advantageous.

In May and June the ability of group A fish to reduce buoyancy was greatest. Seasonal factors contributing to this ability included warmer water temperatures, photoperiod (for large fish only; see above), an increased coefficient of condition, and gonad development. This could be adaptive during the reproductive period of early summer. Fathead minnows can occur in both lotic and lentic waters. As such they can potentially encounter great variation in water velocity in both time and space. The ability to attain the appropriate buoyancy relative to water velocity would broaden the potential areas where spawning could take place and in addition allow fish to maintain territorial position during the reproductive period should there be a sudden change in velocity. In these ways the ability to adjust buoyancy would directly enhance reproductive success.

Acknowledgments

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ALEXANDER, R. MCN. 1959. The densities of Cyprinidae. J. Exp. Biol. 36: 333–340.

1967. Functional design in fishes. Hutchinson and Co. Ltd., London. 160 p.

BEREZAY, G., AND J. H. GEE. 1978. Buoyancy response to changes in water velocity and its function in creek chub (*Semotilus atromaculatus*). J. Fish. Res. Board Can. 35: 295–299.

BONE, Q. 1973. A note on the buoyancy of some lantern fishes (Myctophoidei). J. Mar. Biol. Assoc. U.K. 53: 619–633.

BRAWN, V. 1969. Buoyancy of Atlantic and Pacific herring. J. Fish. Res. Board Can. 26: 2077–2091.

BUTLER, J. L., AND W. G. PEARCY. 1972. Swimbladder morphology and specific gravity of myctophids off Oregon. J. Fish. Res. Board Can. 29: 1145–1150.

DEVRIES, A. L., AND J. T. EASTMAN. 1978. Lipid sacs as a buoyancy adaptation in the Antarctic fish. Nature 271: 352–353.

GEE, J. H. 1968. Adjustment of buoyancy by longnose dace (*Rhinichthys cataractae*) in relation to velocity of water. J. Fish. Res. Board Can. 25: 1485–1496.

1970. Adjustment of buoyancy in blacknose dace, *Rhinichthys atratulus*. J. Fish. Res. Board Can. 27: 1855–1859.

1972. Adaptive variation in swimbladder length and volume in dace, genus *Rhinichthys*. J. Fish. Res. Board Can. 29: 119–127.

1977. Effects of size of fish, water temperature and water velocity on buoyancy alteration by fathead minnows, *Pimephales promelas*. Comp. Biochem. Physiol. 56A: 503–508.

GEE, J. H., AND V. G. BARTNIK. 1969. Simple stream tank simulating a rapids environment. J. Fish. Res. Board Can. 26: 2227–2230.

GEE, J. H., AND P. A. GEE. 1976. Alteration of buoyancy by some Central American stream fishes and a comparison with North American species. Can. J. Zool. 54: 386–391.

GEE, J. H., K. MACHNIAK, AND S. M. CHALANCHUK. 1974. Adjustment of buoyancy and excess internal pressure of swimbladder gases in some North American freshwater fishes. J. Fish. Res. Board Can. 31: 1139–1141.

GIRSA, I. I. 1972. Effect of photoperiod and water temperature on the photoresponse of certain fishes. J. Ichthyol. 12: 554–560.

GROVER, N. C., AND A. W. HARRINGTON. 1966. Stream flow. Measurements, records and their uses. Dover Publications, Inc., New York. 363 p.

HILE, R. 1936. Age and growth of the cisco, *Leucichthys artedi* (Le Sueur) in the lakes of the north-eastern highlands, Wisconsin. Bull. U.S. Bur. Fish 48: 211–317.

HOAR, W. S. 1939. The weight–length relationship of the Atlantic salmon. J. Fish. Res. Board Can. 4: 441–460.

HORAK, D. L. 1966. A densitometric method for determining body fat concentration in fish. Trans. Am. Fish. Soc. 95: 318–320.

KLINGER, S. A. 1978. An investigation of survival mechanisms of three species of fish inhabiting a winterkill lake. M.Sc. thesis, Univ. Wisconsin, Madison, Wisc. 46 p.

LECREN, E. D. 1951. The length–weight relationship and seasonal cycle in gonad weight and condition in the perch (*Perca fluviatilis*). J. Anim. Ecol. 20: 201–219.

LEE, R. F., C. F. PHLEGER, AND M. H. HORN. 1975. Composition of oil in fish bones: possible function in neutral buoyancy. Comp. Biochem. Physiol. 50B: 13–16.

LUOMA, M. E. 1979. Seasonal factors affecting buoyancy attained in still water and in current by fathead minnows, *Pimephales promelas*. M.Sc. thesis, Univ. Manitoba, Winnipeg, Man. 79 p.

MACHNIAK, K., AND J. H. GEE. 1975. Adjustment of buoyancy by tadpole madtom, *Notorus gyrinus* and black bullhead, *Ictalurus melas*, in response to a change in water velocity. J. Fish. Res. Board Can. 32: 303–307.

MACLEOD, J. C. 1967. A new apparatus for measuring maximum swimming speeds of small fish. J. Fish. Res. Board Can. 24: 1241–1252.

MACLEOD, J. C., AND L. L. SMITH. 1966. Effect of pulpwood fiber on oxygen consumption and swimming endurance of the fathead minnow, *Pimephales promelas*. Trans. Am. Fish. Soc. 95: 71–84.

MAGNUSON, J. J., AND D. J. KARLEN. 1970. Visual observation of fish beneath the ice in a winterkill lake. J. Fish. Res. Board Can. 27: 1059–1068.

MILLS, K. H. 1972. Distribution of fishes under the ice in relation to dissolved oxygen, temperature and free, dissolved carbon dioxide in Mystery Lake, Wisconsin. M.Sc. thesis, Univ. Wisconsin, Madison, Wisc. 56 p.

NEAVE, N. M., C. L. DILWORTH, J. G. EALES, AND R. L. SAUNDERS. 1966. Adjustment of buoyancy in Atlantic salmon parr in relation to changing water velocity. J. Fish. Res. Board Can. 23: 1617–1620.

PINDER, L. J., AND J. G. EALES. 1969. Seasonal buoyancy changes in Atlantic salmon (*Salmo salar*) parr and smolt. J. Fish. Res. Board Can. 26: 2093–2100.

SAUNDERS, R. L. 1965. Adjustment of buoyancy in young Atlantic salmon and brook trout by changes in swimbladder volumes. J. Fish. Res. Board Can. 22: 335–352.

SCOTT, W. B., AND E. J. CROSSMAN. 1973. Freshwater fishes of Canada. Fish. Res. Board Can. Bull. 184: 966 p.

TAYLOR, H. F. 1922. Deductions concerning the air bladder and the specific gravity of fishes. Bull. Bur. Fish. 38: 121–126.

Natural Sources and Requirements of Phosphorus for Fishes[1]

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NAKASHIMA, B. S., AND W. C. LEGGETT. 1980. Natural sources and requirements of phosphorus for fishes. Can. J. Fish. Aquat. Sci. 37: 679–686.

The hypothesis that daily consumption of natural prey provides a source of phosphorus sufficient to meet growth and metabolic requirements of fishes is examined. Fish may be capable of utilizing available phosphorus directly from the water; however, this source is negligible in comparison to dietary intake. Experiments were designed to test the effect of different dietary phosphorus levels, chosen to approximate those of natural prey items, on growth of juvenile perch at temperatures encountered during the growing season. Rations were based upon estimates of daily food consumption for perch in Lake Memphremagog. Growth was significantly temperature-dependent and independent of dietary phosphorus levels. Phosphorus:ash ratios of whole fish were not significantly different among diet treatments or between the initiation and termination of the growth experiment. Excretion of phosphorus was positively related to phosphorus intake.

Key words: absorption efficiency, daily ration, growth, Lake Memphremagog, nutrition, *Perca flavescens*, phosphorus budget, yellow perch

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Nous examinons l'hypothèse que la consommation quotidienne de proies naturelles fournit suffisamment de phosphore pour satisfaire aux besoins de croissance et de métabolisme des poissons. Ces derniers sont peut-être capable d'utiliser le phosphore directement de l'eau, mais cette source est négligeable comparée au régime alimentaire. Des expériences ont été conçues en vue de vérifier l'effet de différents niveaux de phosphore alimentaire, choisis de façon à ressembler à ceux des proies naturelles, sur la croissance de jeunes perchaudes aux températures rencontrées durant la saison de croissance. Les rations furent établies d'après estimations de la consommation quotidienne d'aliments par les perchaudes du lac Memphremagog. La croissance est nettement dépendante de la température et indépendante des niveaux de phosphore alimentaire. Les rapports phosphore:cendres de poissons entiers ne diffèrent pas de façon significative d'un régime alimentaire à l'autre ou entre le début et la fin de l'essai de croissance. L'excrétion du phosphore est positivement reliée à l'absorption de cet élément.

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RECENTLY a number of investigators have hypothesized that fish may significantly influence the phosphorus dynamics of freshwater systems (Cooper 1973; Hall et al. 1970; Hurlbert et al. 1972). This influence may be achieved through the continuous excretion of available phosphorus (Lamarra 1975), by the concentrated release of nutrients during decomposition of fish tissue following death (Donaldson 1967; Durbin et al. 1979; Kitchell et al. 1975; Richey et al. 1975), or by the structuring of prey communities (Cooper 1973; Kitchell et al. 1975). However, the importance of nutrient flux through fish, relative to other components of the aquatic system, and the balance between phosphorus intake and storage vs. release in fish remains unclear.

Kitchell et al. (1975) have concluded that the daily consumption of phosphorus by bluegills (*Lepomis macrochirus*) in Lake Wingra, Wisconsin, is insufficient to account for the quantity of phosphorus incorporated during growth. They hypothesized that bluegills actively absorbed phosphorus to supplement their dietary sources. Two important possibilities are raised by this hypothesis. First, if dietary sources of phosphorus are generally inadequate for growth, the majority of the consumed and absorbed phosphorus would be incorporated in tissue. Under such conditions fish populations would act principally as nutrient sinks thereby releasing stored quantities of phosphorus after death (Donaldson 1967; Kitchell et al. 1975; Smith et al. 1977). Secondly, if active uptake of phosphorus is required to support growth, fish may compete with plankton for available phosphorus. A search of the pertinent literature reveals that this hypothesis is at variance with the findings of a number of studies. Smith (1930) reported that the urinary phosphorus of marine fish is exclusively of dietary origin. Coffin et al. (1949) found no evidence of direct uptake of ^{32}P by fish from labeled

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waters of a bog lake and Phillips et al. (1958) reported insignificant uptake of ^{32}P by unfed brook trout (*Salvelinus fontinalis*) held in waters containing high (1 mg·L^{-1}) concentrations of labeled ^{32}P for 96 h.

While the results of these studies do not negate the possibility that fish can actively absorb available phosphorus, they do suggest that dietary sources are generally more than sufficient to meet daily metabolic and growth requirements. If this is so, fish may, through the excretion of excess phosphorus, serve as a source of available phosphorus to the plankton. In this paper we report the results of a study designed to test the hypothesis that phosphorus levels in naturally occurring prey provide a supply of phosphorus sufficient to meet the metabolic and growth demands of fish.

Materials and Methods

We selected juvenile yellow perch (*Perca flavescens*) because of our knowledge of the characteristics of its diet and of its in situ daily ration requirements under differing environmental conditions (B. S. Nakashima and W. C. Leggett, 1978, unpublished data). All fish used were collected with a bag seine on August 25 and 26, 1977 at Prouty Beach, Lake Memphremagog (45°06'N, 72°17'W). The sizes of the fish retained for experimental use were 56.0 ± 2.0 mm (fork length, FL ± 2 SE); 1.76 ± 0.18 g (wet wt ± 2 SE). Following capture we transported our fish in temperature-controlled tanks to the wet laboratory, McGill University, where they were held for ~6 wk in 680-L circular tanks under a 16-h light – 8-h dark photoperiod and at a temperature equal to the lake water temperature at the time of capture (17°C). Brine shrimp were fed to the fish for the first 2 wk after transfer. During this period and subsequent to the elimination of brine shrimp from the diet, we fed Oregon Moist pellets ad libitum for 10 min twice daily.

In mid-October we subdivided all healthy, actively feeding perch into four lots of ~95 fish each and transferred each lot to one of four LSW 700 Living Stream tanks containing water at 17°C. These fish were then acclimated to the final experimental temperatures (12, 15, 18, 21°C) by altering the temperatures in the tanks at the rate of 1°C/5 d. The test temperatures were chosen to encompass the range of temperatures experienced during the period of active growth in Lake Memphremagog (Table 1). Throughout the acclimation and experimental periods, water was exchanged in the tanks at the rate of 0.5 L·h^{-1}.

TABLE 1. Daily ration (% dry food weight per wet body weight) of yellow perch in Lake Memphremagog. The smaller size group (54–78 mm FL) was captured near shore in water <1.1 m and the larger size group (83–150 mm FL) was caught in water 5.0 to 9.5 m deep.

	54–78 mm FL		83–150 mm FL	
	Daily temp. ±2 SE	Ration	Daily temp. ±2 SE	Ration
June	20.9±2.1	2.44	14.5±0.5	0.44
July	23.5±2.0	2.31	20.5±0.6	1.20
Aug.			21.6±0.3	1.50
Sept.	15.3±0.7	1.40	17.5±0.3	0.36
Oct.	12.4±0.8	1.32		

TABLE 2. Dietary composition of the three experimental diets.

Ingredient	% diet 1	2	3
Fish meal	30.00	30.00	30.00
Soybean meal	25.00	27.00	29.00
Dried whey	5.00	5.00	5.00
Blood meal	5.00	5.00	5.00
Fish solubles	5.00	5.00	5.00
Wheat middlings	15.45	7.50	0
Vitamin premix	4.00	4.00	4.00
Trace mineral mix	1.00	1.00	1.00
Propylene glycol	3.00	3.45	3.45
BHT	0.05	0.05	0.05
Choline chloride	0.50	0.50	0.50
Dicalcium phosphate	0	5.00	10.00
Soybean oil	6.00	6.50	7.00
Total	100.00	100.00	100.00
Crude protein (%)	44.5	44.5	44.5
Energy (M.E./kg diet)[a]	2850	2850	2850
Fat (%)	10.7	10.5	10.3
Fiber (%)	2.3	1.8	1.3
Phosphorus (%)	1.12	2.18	3.12
Moisture (%)	6.5	6.6	6.3

[a]M.E., metalizable energy (calculated as total ingested – fecal loss – urine loss) in test conditions.

On December 2 we killed five fish selected at random from each of the four experimental groups for analysis of phosphorus and ash content. The remaining fish in each tank were subdivided into three treatment groups (25–30 fish per treatment group). Each of the three treatment groups maintained at each of four experimental temperatures was then fed one of three experimental diets until the termination of the experiment.

The experimental diets used were specially formulated to investigate the effects of different dietary phosphorus levels on growth of perch. The major dietary determinants of growth (protein, fat, energy) were identical in all three diets. Only the levels of phosphorus varied between diets (Table 2). The dietary phosphorus levels used were chosen to approximate the range of phosphorus found in the spectrum of natural prey (see below) of juvenile and adult perch in Lake Memphremagog (Nakashima and Leggett unpublished data).

Prey type	% phosphorus/dry weight ± 2 SE	Sample size
Cladocerans and copepods[a]	0.7–1.1	
Chironomid larvae	0.93±0.08	10
Crayfish	1.06±0.27	20
0+ *Osmerus mordax*	1.65±0.25	10
Notemigonus crysoleucas	2.51±0.19	10
0+ *Perca flavescens*	3.11±0.13	10

[a]Vijverberg and Frank (1976).

On December 31, when all fish were actively feeding on the experimental diets, the growth experiment was initiated. Each of the 12 treatment groups was fed twice daily at the rate of 1.5 g dry diet/100 g (wet weight) fish·d^{-1}. This

ration was based on known in situ consumption rates for perch in Lake Memphremagog (Table 1). At this ration 100% of the food presented was consumed. All fish were measured and weighed (following anaesthetization with MS222) at the initiation of the experiment and at 14-d intervals thereafter for 20 wk. Ration level was adjusted at each weighing.

To determine changes in body phosphorus and ash levels, five fish were killed from each treatment group at the termination of the experiment. Samples of perch (37–310 mm FL) collected from Lake Memphremagog were analyzed for phosphorus and ash for comparison with experimental fish.

To determine total phosphorus and ash content we removed the contents of the digestive tract, homogenized the entire fish in a Waring Blendor, and dried the homogenate to a constant weight (48–78 h at 80°C). Dried tissue was ground, ashed at 560°C to a constant weight (>12 h), and digested in H_2SO_4. Body phosphorus levels were determined colormetrically using the vanadomolybdate technique (Jackson 1958). No arsenic interference was detected (Johnson 1971).

We estimated food absorption efficiency as

$$\frac{\text{dry weight food} - \text{dry weight feces}}{\text{dry weight food}} \times 100$$

and phosphorus (P) absorption efficiency as

$$\frac{\text{P weight in food} - \text{P weight in feces}}{\text{P weight in food}} \times 100.$$

For these determinations we collected all feces egested by each of the experimental groups over a 72-h period near the termination of the growth experiment. Feces were siphoned from the tanks beginning ~1 h after feeding when maximum egestion occurred. Fish were not starved prior to the collections as is typically done because the effect of starvation on absorption and assimilation is unknown. Phosphorus analyses were performed as described above.

Daily weight and length gains were calculated per fish using the mean length and weight of fish at each measurement (Fig. 1) and dividing the difference by the number of days between measurements. These estimates were then tested for the effect of dietary phosphorus level and temperature on growth with analysis of variance. Significant differences in phosphorus:ash ratios (g phosphorus/100 g ash) between treatments and between experimental and wild perch were tested with the Student–Newman–Keuls test (Sokal and Rohlf 1969).

Results

Growth

Growth was positively related to temperature (Fig. 1) but was unaffected by differences in levels of dietary phosphorus fed at each temperature. Only the main effect of temperature over all diets was significant for growth in length (ANOVA: $F = 86.59$, $P < 0.001$) and in weight (ANOVA: $F = 74.81$, $P < 0.001$). The interaction of diet and temperature and the main effect of diet at all temperatures was not significant ($P > 0.05$). Mortalities were less than 15% and temperatures remained constant during the 20-wk period.

Phosphorus:Ash Ratio

No significant difference was detected in the phosphorus:ash ratios (Table 3) of perch fed different diets within temperature treatments (Student–Newman–Keuls: $\alpha = 0.5$, $k = 4$, $\nu = 12$). Furthermore, final phosphorus:ash ratios of fish in all treatment groups did not differ significantly from pretreatment fish (Student–Newman–Keuls: $\alpha = 0.5$, $k = 4$, $\nu = 16$).

Phosphorus levels of fish sampled directly from Lake Memphremagog were strongly positively related to ash weights. This relationship held for a wide range of body sizes (37–310 mm FL) and from June to October (Fig. 2). Predicted final phosphorus levels of the experimental groups, derived by substitution of grand mean ash weights of experimental fish held at each of the four experimental temperatures into the equation in Fig. 2, did not differ significantly from the experimentally derived values and were as follows:

	Experimental		
Temp., °C	Grand mean ash weight (g)	Grand mean P weight (g)	Predicted P weight (g)
12	0.174	0.033	0.035
15	0.215	0.040	0.043
18	0.375	0.071	0.074
21	0.595	0.111	0.116

Absorption Efficiency

Absorption efficiencies of both food and phosphorus

TABLE 3. Mean phosphorus:ash ratios ±2 SE of juvenile perch at time of capture (Aug. 26), at the introduction of the test diets (Dec. 2), and at the end of the growth experiment (May 19). Sample size in parentheses.

		Temperature treatments			
		12°C	15°C	18°C	21°C
Aug. 26	19.18±0.28 (25)				
Dec. 2		18.30±0.50 (5)	18.31±0.39 (5)	18.54±0.23 (5)	18.55±0.28 (5)
May 19	Diet 1	19.15±0.35 (5)	18.54±0.36 (5)	18.83±0.27 (5)	18.32±0.14 (5)
	Diet 2	19.02±0.31 (5)	18.49±0.29 (5)	18.98±0.25 (5)	18.45±0.14 (5)
	Diet 3	18.68±0.34 (5)	18.74±0.34 (5)	18.97±0.16 (5)	18.71±0.55 (5)
	Grand mean of the three diets	18.95±0.10 (15)	18.59±0.09 (15)	18.93±0.07 (15)	18.50±0.10 (15)

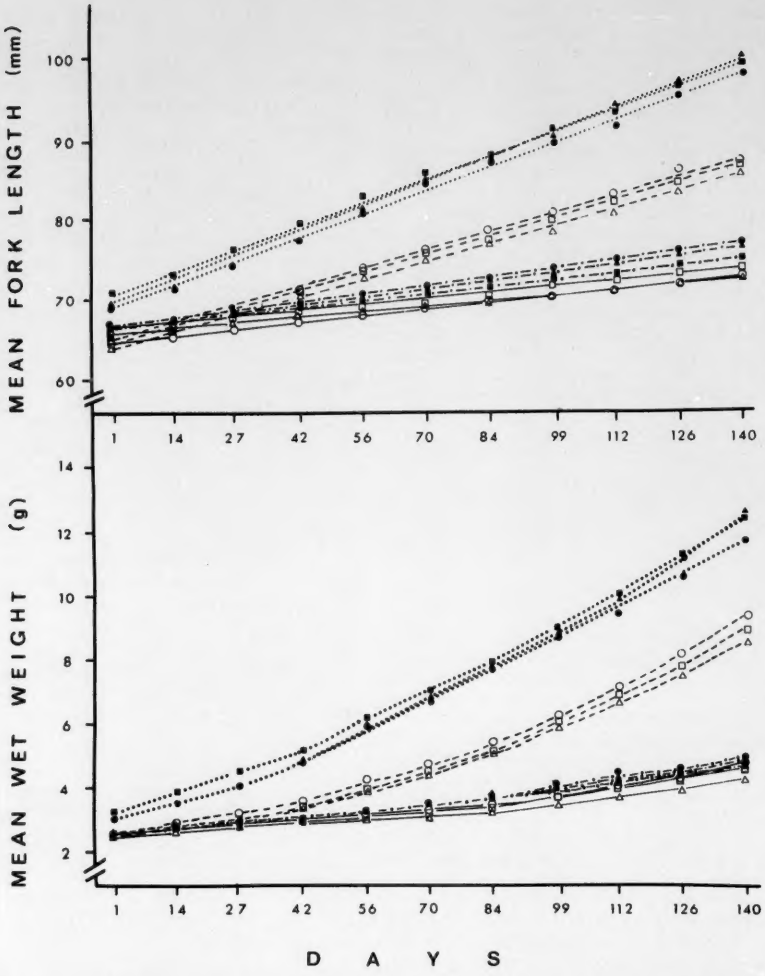

FIG. 1. Mean fork length and mean wet weight of juvenile perch fed test diets containing 1.12% P (circles), 2.18% P (triangles), and 3.12% P (squares) at 12 (—), 15 (-·-·-), 18 (– – –), and 21°C (· · ·).

were inversely related to temperature but were unaffected by differences in dietary phosphorus (Table 4). The absolute quantities of absorbed food and phosphorus were positively related to temperature. Absolute phosphorus absorption was also positively related to levels of dietary phosphorus (Table 4). The absorption efficiencies reported here are believed to be maximum values as some leaching undoubtedly occurred between egestion and collection of feces. Windell et al. (1978) reported that estimates of efficiency changed from 54.7% when determined with materials taken from the lower intestine to 69.4% when determined from feces collected from the experimental tanks 4 h after egestion had taken place. The rate of nutrient loss from the feces may also be positively related to temperature. Because growth and phosphorus:ash ratios were unaffected by dietary phosphorus levels at all temperatures, we conclude that the quantity of phosphorus absorbed in excess of metabolic and structural requirements was excreted as urinary phosphorus.

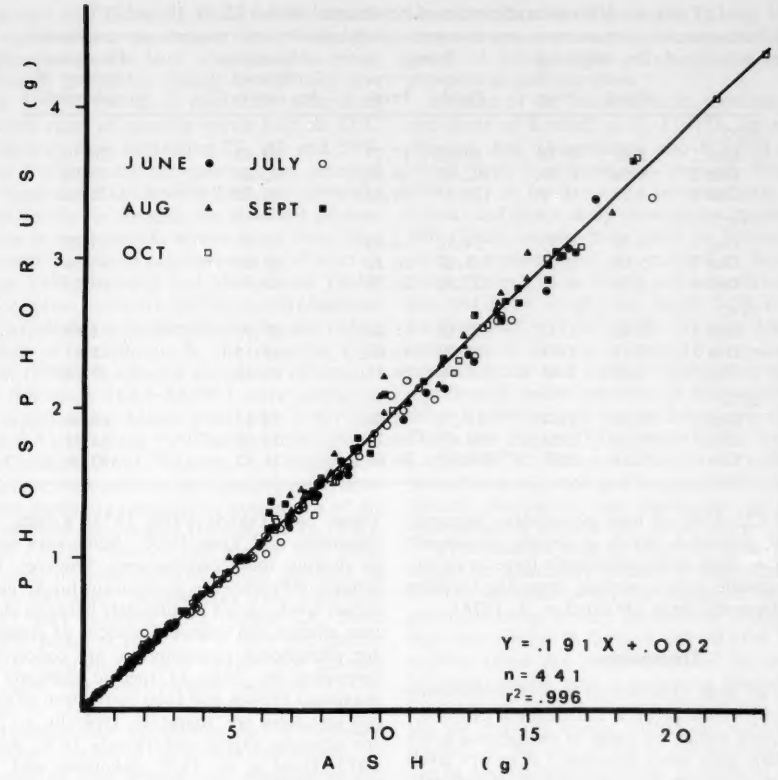

FIG. 2. Relationship between phosphorus and ash content of yellow perch (37–310 mm FL) from Lake Memphremagog. All fish were collected during the 1st wk of each month.

PHOSPHORUS BUDGET

We derived a daily phosphorus budget for perch in the 21°C experimental group. This budget was developed from data collected at the beginning (April 21) and near the end (May 5) of the growth experiment. Mean daily phosphorus intake was derived from the quantity of food consumed and the known phosphorus levels in the diet. Fecal losses were estimated using the phosphorus absorption efficiencies listed in Table 4. Phosphorus incorporated due to growth during the 2-wk interval was estimated by substitution of mean lengths at the beginning and end of the interval of the three treatment groups into the equation:

$$\text{body phosphorus (g)} = -0.2128 + 0.0032\ \text{FL (mm)}, \quad r^2 = 0.91$$

expressing the relationship between body phosphorus and length for the 15 fish killed at the termination of the growth experiment conducted at 21°C. The difference between the initial and final predicted phosphorus levels was attributed to growth.

The quantity of absorbed phosphorus surplus to metabolic and growth needs was estimated as

$$P_{surplus} = P_{ingestion} - P_{growth} - P_{egestion} - P_{maintenance}.$$

Maintenance requirements for phosphorus were estimated by using the value (5.4 mg phosphorus/g body phosphorus·d^{-1}) derived for bluegills by Kitchell et al. (1975). Temperature conditions were similar in the two studies (perch 21°C; bluegill 18–22°C) but our fish were larger (perch 9.2–11.5 g; bluegill 6.5 g). This may have resulted in an overestimate of the maintenance level.

The results of this analysis (Table 5) clearly demonstrate that the quantities of dietary phosphorus consumed with all three diets were more than sufficient to meet the requirements of growth and metabolism.

Estimates of the levels of phosphorus surplus to these

TABLE 4. Absorption efficiencies of experimental diets at 12, 15, 18, and 21°C.

	Dry weight (g)		P (mg)		Absorption efficiency of dry matter (%)	Absorption efficiency of phosphorus (%)
	Food	Feces	Food	Feces		
12°C						
Diet 1	5.01	0.78	59.62	17.77	84.43	70.19
Diet 2	4.68	0.83	102.02	28.03	82.26	72.52
Diet 3	4.14	0.79	129.17	36.02	80.92	72.11
15°C						
Diet 1	4.59	0.33	54.62	8.84	92.82	83.82
Diet 2	5.04	0.46	109.87	16.52	90.87	84.96
Diet 3	4.08	0.45	127.30	24.79	88.97	80.53
18°C						
Diet 1	8.43	1.52	100.32	25.44	81.97	74.64
Diet 2	7.11	1.34	155.00	43.60	81.15	71.87
Diet 3	7.20	1.43	224.64	60.21	80.14	73.20
21°C						
Diet 1	9.69	2.44	115.31	34.64	74.82	69.96
Diet 2	9.15	2.58	199.47	70.72	71.80	64.55
Diet 3	9.81	2.46	306.07	90.66	74.92	70.38

requirements (23–53% of total phosphorus ingested) were positively related to levels of dietary phosphorus and, at all three diets, were sufficiently large to be unaffected by possible errors resulting from the leaching of nutrients from the feces (Windell et al. 1978).

Discussion

The results of these experiments clearly demonstrate that growth of juvenile perch is independent of dietary phosphorus levels within the range of phosphorus levels found in natural prey when consumed at in situ levels of daily ration. Moreover, the constancy of phosphorus: ash ratios between treatment groups and at different temperatures indicates that all dietary phosphorus levels were adequate to meet fully the requirements of both bone and scale formation and routine metabolism. Phosphorus absorbed in excess of these requirements must, therefore, be excreted. These findings were not unexpected because the dietary phosphorus levels of natural prey were within, or greater than, the range of minimum phosphorus levels (0.6–1.3%) known to be required for maximum growth in several fish species (Andrews et al. 1973; Dove et al. 1976; Ketola 1975; Ogino and Takeda 1976, 1978; Reinïtz et al. 1978; Sakamoto and Yone 1978). Some care must be taken in making these comparisons, however, because significant differences in phosphorus intake could occur if ration levels varied significantly between studies. Nutrition studies, the principal sources of comparative data for phosphorus consumption, are concerned with determining the levels of specific elements required to maximize growth and food conversion efficiency. Feeding schedules are, therefore, typically ad libitum (see, for example, Ogino and Takeda 1976; Andrews et al. 1973; Dove et al. 1976; Sakamoto and Yone 1978; Ketola 1975). In our experiments perch held at 12, 15, and 18°C were observed to be satiated by our feeding regime, but those held at 21°C were not. For this reason the 1.5% (dry weight) daily ration consumed by perch held at 21°C is believed to be closer to the lower limit of diets fed in other studies. We have no information concerning the digestibility of phosphorus in natural diets. The levels of phosphorus in fishes consumed by perch are overestimates of available dietary phosphorus because scales and bones are resistant to digestion processes (Molnár and Tölg 1962). Therefore, our dietary phosphorus level of 3.12% dry weight represents concentrations greater than would be nutritionally available in natural prey. In spite of these restrictions on phosphorus ingestion at 21°C, no effect of dietary phosphorus on growth or phosphorus:ash ratios was detected and the quantity of phosphorus ingested was greater than the combined requirements for growth and maintenance.

The possibility exists that the similarity in growth and phosphorus absorption rates observed in our experiments resulted from the regulatory effects of nutritional factors other than phosphorus. We strongly believe, however, that this was not the case. This conclusion is

TABLE 5. Daily phosphorus budget (mg) for yellow perch at 21°C fed diets of 1.12, 2.18, and 3.12% phosphorus content.

	P intake	P egestion (feces)	P growth	P maintenance[a]	P surplus
Diet 1	1.58	0.48	0.31	0.43	0.36
Diet 2	3.52	1.25	0.31	0.51	1.45
Diet 3	5.45	1.61	0.44	0.50	2.90

[a]Estimated from Kitchell et al. (1975).

based on the fact that the composition of our experimental diet was similar to the Oregon Moist Pellet diet except for phosphorus. The level of protein, the major element affecting growth in fishes, in our diet was 44.5%. Huh (1975) found no significant differences between growth rates of juvenile perch held at 22°C when administered diets containing 27, 40, and 50% protein. The requirements for calcium, an essential mineral for bone and scale development, are generally met by active uptake via the gills, the dissolved calcium concentrations in most natural waters being more than adequate to meet nutritional requirements (McCay et al. 1936; Phillips 1959; Podoliak and McCormick 1966). High dietary calcium levels can inhibit growth (Andrews et al. 1973) by binding with phosphorus thereby reducing absorption of phosphorus in the digestive tract (Phillips et al. 1958). Phosphorus absorption efficiencies observed in this study (64.5–84.9%) were similar to efficiencies reported for brook trout (58–71%) fed capsules with 0.5 and 2.0 mg ^{32}P (Podoliak 1961; Podoliak and McCormick 1966). Vitamin D_3 is required to promote calcium absorption in the digestive tract; however, very low levels are required because much of the calcium is absorbed via the gills (Lovell and Li 1978). Launer et al. (1978) reported that growth and phosphorus retention of pond-cultured catfish required no vitamin D_3 in their diets. Natural prey were consumed and this may have supplied some vitamin D_3. Food absorption efficiencies of 71.8–74.9% were also similar to efficiencies reported by Huh (1975) for juvenile perch (74.3–86.7%) and juvenile walleye (74.3–84.5%) held at 22°C. Thus we conclude that nutritional factors other than phosphorus had a negligible influence on the outcome of the growth experiment.

Our observation that growth and phosphorus:ash ratios of juvenile perch were unaffected by differences in dietary phosphorus within the normal range of phosphorus levels found in natural prey when consumed at in situ levels of daily ration supports our hypothesis that natural dietary levels of phosphorus are adequate to meet all metabolic and structural requirements for phosphorus. This conclusion is further supported by the results of our phosphorus budget analysis for perch in the 21°C experimental group which indicated that a significant proportion of the total phosphorus ingested is surplus to the requirements for growth and maintenance and is excreted along with phosphorus used for maintenance functions, presumably via the urine. Some caution must be exercised in interpreting the magnitude of this excreted fraction because of difficulties in determining the maintenance requirements for phosphorus and our assumption that maintenance values derived for bluegills can be applied to perch. In addition, differences in the average size of perch in our experiments and bluegills in the study conducted by Kitchell et al. (1975) would positively bias the estimate of phosphorus maintenance used, and lead to an underestimate of surplus phosphorus. The finding that dietary phosphorus levels <0.6% frequently result in abnormal bone development (Ogino and Takeda 1976) further supports our argument by demonstrating the inadequacy of active uptake of phosphorus to supplement phosphorus-deficient diets.

Because of the inconsistencies between our findings and those of Kitchell et al. (1975), we examined the original data (Magnusson and Kitchell 1971) from which their hypothesis was derived. We believe the difference to be due to an underestimate of the daily ration, and hence daily phosphorus ingestion, of bluegills. Daily rations from May to October, estimated using a derivation of the method of Bajkov (1935), ranged from 0.96 to 3.20% wet stomach content weight per wet body weight for small, 0.59 to 1.71% for medium, and 0.28 to 1.63% for large bluegills. These estimates are close to estimated maintenance levels for juvenile perch and walleye (Kitchell et al. 1977) and significantly below estimates of daily ration for several other freshwater and marine fish species (Clarke 1978; Doble and Eggers 1978; Healey 1979; Nakashima and Leggett 1978). This would lead to an underestimate of phosphorus ingestion and hence to their conclusion that dietary phosphorus was inadequate to meet the requirements of growth and metabolism.

While caution must be exercised in extrapolating the results of laboratory studies to field situations (Kerr 1971), we believe that the design of our growth experiment using dietary phosphorus levels in test diets that were similar to those of natural prey, feeding daily rations within the range estimated for perch in Lake Memphremagog, and maintaining temperature conditions similar to temperatures experienced by perch during the growing season, justify our conclusion that an analysis of the role of fish in the recycling of nutrients can be approached directly by monitoring dietary input, growth, and excretion without concern for the possible complicating influence of active uptake from aquatic sources.

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ANDREWS, J. W., T. MURAI, AND C. CAMPBELL. 1973. Effects of dietary calcium and phosphorus on growth, food conversion, bone ash and hematocrit levels of catfish. J. Nutr. 103: 766–771.

BAJKOV, A. D. 1935. How to estimate the daily food consumption of fish under natural conditions. Trans. Am. Fish. Soc. 65: 288–289.

CLARKE, T. A. 1978. Diel feeding patterns of 16 species of mesopelagic fishes from Hawaiian waters. U.S. Natl. Mar. Fish. Serv. Fish. Bull. 76: 495–513 p.

COFFIN, C. C., F. R. HAYES, L. H. JODREY, AND S. G. WHITEWAY. 1949. Exchange of materials in a lake as studied by the addition of radioactive phosphorus. Can. J. Res. Sec. D27: 207–222.

COOPER, D. C. 1973. Enhancement of net primary productivity by herbivore grazing in aquatic laboratory microcosms. Limnol. Oceanogr. 18: 31–37.

DOBLE, B. D., AND D. M. EGGERS. 1978. Diel feeding chronology, rate of gastric evacuation, daily ration, and prey selectivity in Lake Washington juvenile sockeye salmon (*Oncorhynchus nerka*). Trans. Am. Fish. Soc. 107: 36–45.

DONALDSON, J. R. 1967. The phosphorus budget of Iliamna Lake, Alaska, as related to the cyclic abundance of sockeye salmon. Ph.D. thesis, Univ. Washington, Seattle, Wash. 141 p.

DOVE, G. R., O. W. TIEMIER, AND C. W. DEYOE. 1976. Effects of three diets on growth and mineral retention of channel catfish fingerlings. Trans. Am. Fish. Soc. 105: 481–485.

DURBIN, A. G., S. W. NIXON, AND C. A. OVIATT. 1979. Effects of the spawning migration of the alewife, *Alosa pseudoharengus*, on freshwater ecosystems. Ecology 60: 8–17.

HALL, D. J., W. E. COOPER, AND E. E. WERNER. 1970. An experimental approach to the production dynamics and structure of freshwater animal communities. Limnol. Oceanogr. 15: 839–928.

HEALEY, M. C. 1979. Detritus and juvenile salmon production in the Nanaimo estuary: I. Production and feeding rates of juvenile chum salmon (*Oncorhynchus keta*). J. Fish. Res. Board Can. 36: 488–496.

HUH, H. T. 1975. Bioenergetics of food conversion and growth of yellow perch (*Perca flavescens*) and walleye (*Stizostedion vitreum vitreum*) using formulated diets. Ph.D. thesis, Univ. Wisconsin, Madison, Wisc. 196 p.

HURLBERT, S. H., J. ZEDLER, AND D. FAIRBANKS. 1972. Ecosystem alteration by mosquitofish (*Gambusia affinis*) predation. Science 175: 639–641.

JACKSON, M. L. 1958. Soil chemical analysis. Prentice-Hall Inc., Englewood Cliffs, N.J. 498 p.

JOHNSON, D. L. 1971. Simultaneous determinations of arsenate and phosphate in natural waters. Environ. Sci. Technol. 5: 411–414.

KERR, S. R. 1971. Analysis of laboratory experiments on growth efficiency of fishes. J. Fish. Res. Board Can. 28: 801–808.

KETOLA, H. G. 1975. Requirements of Atlantic salmon for dietary phosphorus. Trans. Am. Fish. Soc. 104: 548–551.

KITCHELL, J. F., J. F. KOONCE, AND P. S. TENNIS. 1975. Phosphorus flux through fishes. Int. Ver. Theor. Angew. Limnol. Verh. 19: 2478–2484.

KITCHELL, J. F., D. J. STEWART, AND D. WEININGER. 1977. Applications of a bioenergetics model to yellow perch (*Perca flavescens*) and walleye (*Stizostedion vitreum vitreum*). J. Fish. Res. Board Can. 34: 1922–1935.

LAMARRA, V. A. JR. 1975. Digestive activities of carp as a major contributor to the nutrient loading of lakes. Int. Ver. Theor. Angew. Limnol. Verh. 19: 2461–2468.

LAUNER, C. A., O. W. TIEMIER, AND C. W. DEYOE. 1978. Effects of dietary addition of vitamins C and D_3 on growth and calcium and phosphorus content of pond-cultured channel catfish. Prog. Fish Cult. 40: 16–20.

LOVELL, R. T., AND Y.-P. LI. 1978. Essentiality of vitamin D diets of channel catfish (*Ictalurus punctatus*). Trans. Am. Fish. Soc. 107: 809–811.

MAGNUSSON, J. J., AND J. F. KITCHELL. 1971. Energy-nutrient flux through fishes—Lake Wingra. Eastern Deciduous Forest Biome Rep. 71–58: 41 p.

MCCAY, C. M., A. V. TUNISON, M. CROWELL, AND H. PAUL. 1936. The calcium and phosphorus content of the body of the brook trout in relation to age, growth, and food. J. Biol. Chem. 114: 259–263.

MOLNÁR, G., AND I. TÖLG. 1962. Relation between water temperature and gastric digestion of largemouth bass (*Micropterus salmoides* Lacépède). J. Fish. Res. Board Can. 19: 1005–1012.

NAKASHIMA, B. S., AND W. C. LEGGETT. 1978. Daily ration of yellow perch (*Perca flavescens*) from Lake Memphremagog, Quebec–Vermont, with a comparison of methods for in situ determinations. J. Fish. Res. Board Can. 35: 1597–1603.

OGINO, C., AND H. TAKEDA. 1976. Mineral requirements in fish—III. Calcium and phosphorus requirements in carp. Nippon Suisan Gakkaishi 42: 793–799.

1978. Requirements of rainbow trout for dietary calcium and phosphorus. Nippon Suisan Gakkaishi 44: 1019–1022.

PHILLIPS, A. M. JR. 1959. The known and possible role of minerals in trout nutrition and physiology. Trans. Am. Fish. Soc. 88: 133–135.

PHILLIPS, A. M. JR., H. A. PODOLIAK, R. F. DUMAS, AND R. W. THOESEN. 1958. The nutrition of trout. Fish. Res. Bull. 22. Cortland Hatchery Rep. 27: 87 p.

PODOLIAK, H. A. 1961. Relation between water temperature and metabolism of dietary phosphorus by fingerling brook trout. Trans. Am. Fish. Soc. 90: 398–403.

PODOLIAK, H. A., AND J. H. MCCORMICK. 1966. Distribution of dietary phosphate to the acid soluble fraction of the muscle and viscera of brook trout. Fish. Res. Bull. 30. Cortland Hatchery Rep. 35: 14–24 p.

REINITZ, G. L., L. E. ORME, AND F. N. HITZEL. 1978. Phosphorus requirement for rainbow trout. Feedstuffs 50: 17.

RICHEY, J. E., M. A. PERKINS, AND C. R. GOLDMAN. 1975. Effects of kokanee salmon (*Oncorhynchus nerka*) decomposition on the ecology of a subalpine stream. J. Fish. Res. Board Can. 32: 817–820.

SAKAMOTO, S., AND Y. YONE. 1978. Effect of dietary phosphorus level on chemical composition of Rea Sea bream. Nippon Suisan Gakkaishi 44: 227–229.

SMITH, E. A. H., C. I. MAYFIELD, AND P. T. S. WONG. 1977. Colonization and decomposition of fish bone material in natural and synthetic aqueous solutions. J. Fish. Res. Board Can. 34: 2176–2184.

SMITH, H. W. 1930. The absorption and excretion of water and salts by marine teleosts. Am. J. Physiol. 93: 480–505.

SOKAL, R. R., AND F. J. ROHLF. 1969. Biometry. W. H. Freeman and Company, San Francisco. 776 p.

VIJVERBERG, J., AND T. H. FRANK. 1976. The chemical composition and energy contents of copepods and cladocerans in relation to their size. Freshwater Biol. 6: 333–345.

WINDELL, J. T., J. W. FOLTZ, AND J. A. SAROKON. 1978. Methods of fecal collection and nutrient leaching in digestibility studies. Prog. Fish Cult. 40: 51–55.

Estimating Parameters in a Flexible Growth Equation, the Richards Function[1]

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Two methods are presented for estimating parameters in the Richards function, a generalized growth equation. Both methods use typical mark–recapture data: initial and final size separated by a time interval. Three examples are presented. Data on the sea urchin, *Diadema setosum*, is best fit with Method I and tree seedling (*Acer pseudoplatanus*) data by Method II. Cisco (*Coregonus artedii*) growth is described about equally well by either method. The two methods could be applied to population growth in numbers as well as individual growth in size.

Key words: Richards function, Brody–Bertalanffy, logistic, growth

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Nous présentons deux méthodes d'estimation des paramètres dans la fonction de Richards, une équation de croissance généralisée. Les deux méthodes font appel aux données typiques de recaptures des marques : taille initiale et taille finale séparées par un intervalle de temps. Nous donnons trois exemples. La méthode I donne la meilleure adaptation des données sur l'oursin de mer *Diadema setosum*, alors que dans le cas des données sur de jeunes arbres (*Acer pseudoplatanus*), c'est la méthode II qui donne les meilleurs résultats. L'une ou l'autre méthode décrivent également bien la croissance du cisco (*Coregonus artedii*). Les deux méthodes pourraient être appliquées à la croissance numérique d'une population ainsi qu'à l'augmentation de taille des individus.

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STUDIES of growth, either of individuals or populations, are fundamental to both theoretical and applied ecology. The two most commonly used growth models are the Brody–Bertalanffy for individual growth in size (see Ricker 1975 for a review of literature) and the logistic for population growth in numbers (see for example, Smith 1974 or any current general ecology text). The actual shape of a growth curve frequently does not conform to either of these models, yet both functions are widely used. For theoretical considerations this is of little consequence but for calculation of yield or production or for use in estimating mortality rates from individual growth and population size structure (Ebert 1973; Van Sickle 1977a), the best growth model possible is required. The Richards function (Richards 1959) is a flexible growth model that includes both the Brody–Bertalanffy and the logistic equations. It has not, however, been widely used. Part of the lack of use of the Richards function probably is due to a lack of information that closely ties parameter estimation to existing ways of gathering field data.

Existing procedures for estimating parameters in the Richards function (Richards 1959; Nelder 1961; Causton 1969; Causton et al. 1978; Pienaar and Thomson 1973; Johnson et al. 1975) require knowing both size and age of organisms. The purpose of this note is to present two new methods for estimating parameters of the Richards function using typical mark–recapture data, i.e. initial and final sizes separated by a known time interval. Age of individuals does not have to be known.

The Richards function (Richards 1959; Fletcher 1975) can be written

$$(1) \quad S_t = S_\infty(1 - be^{-Kt})^{-n}$$

in which S_t is size of an individual at time t; S_∞ is asymptotic size; b is a scaling parameter equal to $(S_\infty{}^{(-1/n)} - S_0{}^{(-1/n)})/S_\infty{}^{(-1/n)}$ with S_0 equal to size at birth, germination, settlement, etc.; K is a growth rate constant; and n is a shape parameter. When $n = -1$ and measurements are linear, the function is the Brody–Bertalanffy equation. If measurements are weights and $n = -1$, the equation is the monomolecular model. When n is $+1$ the function is the logistic and as $|n| \to \infty$ the function approaches the Gompertz equation.

Method I

Equation 1 can be rewritten as

$$(2) \quad S_t{}^{(-1/n)} = S_\infty{}^{(-1/n)}(1 - be^{-Kt})$$

in which form it can be manipulated in the same manner

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as the Brody–Bertalanffy equation was by Ford (1933) and Walford (1946). Equation 2 can be rearranged further to

$$(3)\quad S_\infty^{(-1/n)} - S_t^{(-1/n)} = S_\infty^{(-1/n)} be^{-Kt}.$$

If size is measured when an individual is t time units old its size is described by eq (2). If it is measured T time units later its size is

$$(4)\quad S_{t+T}^{(-1/n)} = S_\infty^{(-1/n)} - S_\infty^{(-1/n)} be^{-Kt}e^{-KT}.$$

From eq (3) $(S_\infty^{(-1/n)} - S_t^{(-1/n)})$ can be substituted for $S_x^{(-1/n)}{}_{be}{}^{-Kt}$ so eq (4) becomes

$$(5)\quad S_{t+T}^{(-1/n)} = S_\infty^{(-1/n)} - (S_\infty^{(-1/n)} - S_t^{(-1/n)})e^{-KT}$$

from which a suitable linear equation can be formed

$$(6)\quad S_{t+T}^{(-1/n)} = S_\infty^{(-1/n)}(1 - e^{KT}) + S_t^{(-1/n)}e^{-KT}$$

Note that b has dropped out. The parameter b does nothing more than position the growth curve on the time axis. Lack of specific information on size at some particular age does not affect estimation of other parameters. If pairs of size measurements are obtained that are separated by some time interval T, a graph of $S_{t+T}^{(-1/n)}$ vs. $S_t^{(-1/n)}$, which is called a Walford plot for Brody–Bertalanffy growth (i.e. $n = -1$) (Ricker 1975), has a slope m, equal to e^{-KT} and a y-intercept, C, equal to $S_\infty^{(-1/n)}(1 - e^{-KT})$; therefore

$$(7)\quad K = \ln(m)/T$$

and

$$(8)\quad S_\infty = (C/(1 - m))^{-n}.$$

The parameter n is selected to minimize the sums of squares of error (SSE) around the calculated curve

$$(9)\quad \text{SSE} = \sum_{t=1}^{N} [Y_t - (mX_t + C)^{-n}]^2$$

where $Y = S_{t+T}$ and $X = S_t^{(-1/n)}$.

SSE in eq (9) is the same as the sums of squares term in the likelihood function given by Sprott and Kalbfleisch (1965) and used by Pienaar and Thomson (1973).

Minimization of SSE is done by trial and error. The search is in two parts, first for negative values of n and then for positive values. The significant steps in the fitting program are establishing initial values, transforming the data, and performing a least-squares regression, using the regression parameters to calculate SSE and then searching for a transformation that produces the smallest SSE.

Important initial values are a "saved residual sums of squares" that is set equal to 10 000, an initial value for the shape parameter n that is used to transform the data ($n = -25$) and an initial increment ($D = 5$) that will change the value of the shape parameter after each loop.

Data are transformed so each size pair (initial and final sizes) are changed by the shape parameter n:

$$(10)\quad TS_1 = S_1^{(-1/n)}$$

$$(11)\quad TS_2 = S_2^{(-1/n)}.$$

The regression is TS_2 vs. TS_1 (eq (6)) and because both the x and y values are subject to an equal amount of variability, a geometric mean (GM) functional regression is used with eq (6) to calculate slope and intercept (Ricker 1973). With a GM functional regression, the slope m in eq (7) and eq (8) must be replaced by $v = m/r$ where r is the product–moment correlation coefficient. Also, C must be replaced by C' by solving the equation $C' = \bar{y} - v\bar{x}$; $\bar{y}$ is the mean of TS_2 and $\bar{x}$ is the mean of TS_1.

The regression parameters v and C' are used in eq (9) to estimate SSE. To begin, $n = -25$ and the calculated value of SSE is compared with the "saved residual sums of squares," SSSE. If SSE is smaller, its value replaces the value in SSSE and the value of n is saved.

Initially, there is a loop of five iterations which change by increments of 5 ($n = -25, -20, -15, -10$, and -5). After these five iterations, the saved value of n, that is the value of n that produced the smallest SSE, is decremented by 5, and 10 iterations are performed in the loop with increments of 1. This procedure ensures that the value of n that produces the smallest SSE is bracketed on either side by one entire interval of 5. For example, if the saved value of n is -10 after the first five iterations, then the domain for the next set of iterations is -15 to -5 and this domain is explored using increments of 1.

After 10 iterations, the saved value of n is decremented by 1, and 10 more iterations are performed using increments of 0.2 (i.e. ⅕). Once again this procedure brackets the saved value of n by one increment on either side. If the saved value of n is $n = -7$, then the domain of the next 10 iterations is from -8 to -6. The bracketing process is continued for seven loops. Each loop changes the increment (D) by dividing by 5 so the final loop uses an increment of 0.00032. A total of 65 iterations may be performed (5 iterations for the first loop and 10 for each of the remaining six loops). The final value of n that provides the smallest SSE overall is saved together with its SSE. The entire process is reinitiated with positive values of n ($n = +25$) and, potentially, can continue for 65 more iterations; however, iterations are terminated if the saved value of $|n|$ is 25 for the first five iterations.

The smallest value for SSE using negative values of n is compared with the smallest value of SSE using positive values of n and the value of n that gives the smaller SSE is selected as the best global estimate of the shape parameter. If the best estimate of $|n|$ is 25, then the Gompertz curve is taken as the best fit.

It must be made clear that the trials include a limited number of values of n — no more than 130 values. Because the smallest increment is 0.00032, there are a total possible number of 156 250 values that could be tried (50/0.00032).

This rather cumbersome search procedure was selected because: (1) more sophisticated procedures frequently end at false minima or are unstable and (2) there is a discontinuity at $n = 0$ that precludes dealing with both sides of zero at the same time. Problems of false minima and instability are referred to by Causton (1969), Causton et al. (1978), Pienaar and Thomson (1973), and Davis and Ku (1977) in fitting the Richards function.

Method II

Causton (1969) presents a method for obtaining initial estimates of n, K, and S_∞ in his procedure which requires both age and size. His technique for obtaining initial estimates can be modified for use with mark–recapture data. Causton shows that a regression of growth rate (R) vs. size ($S^{(-1/m)}$ can be used to estimate parameters. Growth rate (R) is

$$(12)\quad R = (\ln S_{t+T} - \ln S_t)/T.$$

The value of R would best apply to an individual age t +

$0.5T$ but without knowing growth parameters it is not possible to estimate $S_{t+0.5T}{}^{(-1/n)}$ directly. However, if T is short relative to the total time required for full growth, then a reasonable approximation to size at $t + 0.5T$ is

$$(13)\quad S_{t+0.5T}{}^{(-1/n)} \simeq (S_{t+T}{}^{(-1/n)} + S_t{}^{(-1/n)})/2 = S^{(-1/n)}.$$

As Causton shows:

$$(14)\quad R = a + dS^{(-1/n)}$$

also,

$$(15)\quad K = a/n$$

and,

$$(16)\quad S_\infty = (-nK/d)^n.$$

As in Method I, n is selected by trial and error to minimize SSE in eq (9). Minimization is done using a Walford plot so the two methods can be compared and the parameters selected that give the smallest SSE regardless of method. Values of m and C must be determined from values of a and d to use eq (9):

$$(17)\quad m = e^{-aT/n}$$

$$(18)\quad C = -d(1 - m)/Kn.$$

Method II, which plots R vs. $S^{(-1/n)}$, is the same procedure proposed by Bayley (1977) for estimating parameters of the Brody–Bertalanffy curve. Bayley plotted R vs. $1/S$ which is the same as using a value of $n = -1$, the appropriate constant in Richards function for Brody–Bertalanffy.

Bayley (1977) argued that for the Brody–Bertalanffy model, R. vs. S^{-1} should use a predictive rather than a GM functional regression because S^{-1} "should normally be much more accurate and subject to less natural variability than" R. I have followed Bayley's suggestion and have used a predictive regression with Method II.

For either method, parameter b cannot be determined using mark–recapture data; at least one age and size estimate is required. Size at birth, settlement, or germination often can be approximated even if size and age data are not available. I have added the parameter n to Fabens' (1965) formulation for estimating b

$$(19)\quad b = \frac{\Sigma[e^{-Kt'}(S_\infty{}^{(-1/n)} - S_{t'}{}^{(-1/n)})]}{S_\infty{}^{(-1/n)}\Sigma(e^{-Kt'})^2}$$

where $S_{t'}$ is size at a known age t'. As formulated by Fabens, one or more values of size at a known age can be used to estimate b. If a single value of size at $t = 0$ is used, then eq (19) reduces to

$$(20)\quad b = (S_\infty{}^{(-1/n)} - S_0{}^{(-1/n)})/S_\infty{}^{(-1/n)}.$$

It should be noted that the calculation of b using eq (19) or (20) provides the necessary sign change in the Richards formulation (shown by Causton 1969) as n goes from negative to positive. The sign of b is positive for negative values of n and negative for positive values of n.

Three examples will be presented which will show one set of data that is fit better using Method I, one set that is fit better by Method II, and one set that gives similar results with either method. Comparisons of methods are made using a likelihood ratio.

Pienaar and Thomson (1973) used a likelihood function obtained from Sprott and Kalbfleisch (1965) to determine the influence of small changes in parameters of the Richards function on the fit of the regression line to the data. I have used this likelihood function to compare the parameters estimated from Methods I and II as well as parameters from other programs. The function given by Sprott and Kalbfleisch is

$$(21)\quad L(\theta) = (1/\sigma)^N \exp[-1(2\sigma^2)\Sigma(y_i - \beta x_i)^2]$$

in which

$$(22)\quad \sigma^2 = \mathrm{SSE}/N$$

and

$$(23)\quad \Sigma(y_i - \beta x_i)^2 = \mathrm{SSE} \quad \text{(from eq (9))}$$

N = number of observations.

Substituting from 22 and 23 into 21 yields

$$(24)\quad L(\theta) = N^{N/2} \exp(-N/2)\mathrm{SSE}^{-N/2}.$$

A constant c can be defined to collect terms that do not contain the parameters, n, K, and S_∞

$$(25)\quad c = N^{N/2} \exp(-N/2)$$

so

$$(26)\quad L(\theta)/c = \mathrm{SSE}^{-N/2}$$

which is the form used by Pienaar and Thomson (1973). Comparison of different methods or programs is done by a likelihood ratio $R(\theta)$

$$(27)\quad R(\theta) = L(\hat{\theta})/L(\theta)$$

in which $L(\hat{\theta})/c$ is the likelihood function that produced the maximum value, that is, had the smallest SSE.

Examples

Example 1

Figure 1 shows length of half-pyramids (jaw elements) of 55 individuals of a sea urchin, *Diadema setosum*, from Zanzibar. Size after 1 yr (S_{t+1}) is plotted vs. the original

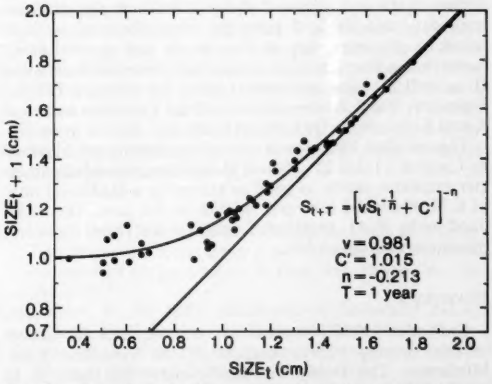

FIG. 1. Growth of half-pyramids (jaw elements) of a sea urchin, *Diadema setosum*. Size after 1 yr is plotted vs. original size. Straight line is the line of zero growth. Parameters for the fitted curve are from Method I.

TABLE 1. Comparison of Methods I and II for estimating parameters of the Richards function. Data are for a sea urchin, *Diadema setosum*, from Zanzibar, and are shown in Fig. 1. $N = 55$. $T = 1$ year.

	Method I	Method II
n	−0.213	−0.578
K	0.019	0.578
S_∞	2.328 cm	1.587 cm
SSE	0.12345	0.31314
$L(\theta)/c$	9.638×10^{24}	7.365×10^{13}
$R(\theta)$	1.0	7.64×10^{-12}

size (S_t) which was determined by tagging individuals with tetracycline. The points are scattered around a curve which shows that small individuals grow very rapidly and that large urchins continue to grow for a long time but at a slow and nearly constant rate (a slope of 1 would be a constant rate).

As shown in Table 1, the two methods do not yield the same minimum residual sums of squares, SSE. Method I gives the smaller SSE and the likelihood ratio $R(\theta)$ is 7.64×10^{-12}. The parameters determined using Method II are much less likely to estimate correctly S_{t+1} from S_t than are the parameters estimated using Method I.

The scaling parameter b is not included in minimizing SSE; however, if information on size and age are available, b can be determined using eq (19) after the parameters n, K, and S_x have been estimated.

A newly settled urchin has a jaw length of about 0.01 cm so $b = 1.00$. The growth equation for a half-pyramid is

$$(28)\quad S_t = 2.33(1 - e^{-0.018t})^{-0.213}.$$

Size is given in centimetres and age in years.

EXAMPLE 2

Causton (1969) gives age and weight data for a sycamore (*Acer pseudoplatanus*) seedling. The seedling showed exponential growth for most of the season with a very sharp decline at the end. Figure 2 shows a Walford plot of Causton's data and Table 2 gives the comparison of methods based on the regression of S_{t+2} vs. S_t and eq (9). Comparisons can be made using parameters from Methods I and II as well as using parameters given by Causton (1969). Equations 7 and 8 were used to change Causton's values of K and S_x into slope (m) and intercept (C) for use in eq (9).

The smallest SSE was produced by parameters obtained by Causton (Table 2). Method II, however, provided parameter estimates nearly as good as shown by a likelihood ratio of 0.78. Method I is clearly inferior in this case. The likelihood ratio $R(\theta)$ (compared with results from Causton's parameters) is only 0.001.

EXAMPLE 3

Carlander (1950) presents size and age data for ciscoes or lake herring (*Coregonus artedii*) in Vermilion Lake, Minnesota. The Brody–Bertalanffy curve has been fit to these data by Ricker (1975) and Bayley (1977) using several approaches. In addition to using Methods I and II, parameters were estimated by a nonlinear least squares program using Marquardt's algorithm. The program is part of

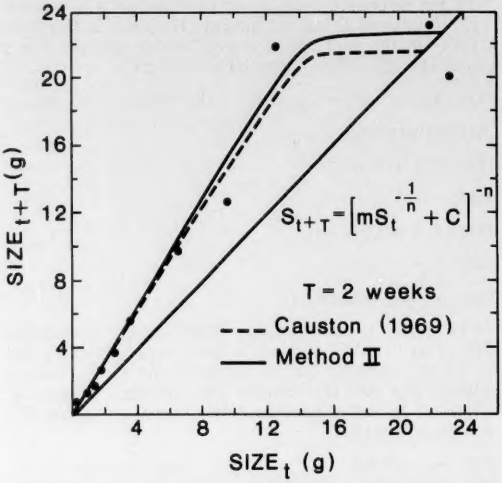

FIG. 2. Growth of a tree seedling (*Acer pseudoplatanus*). Data from Causton (1969). Causton's program gives the smaller SSE. The likelihood ratio $R(\theta)$ is 0.78.

the Statistical Analysis System (SAS) package. A new version of Causton's program (Causton et al. 1978) also was tried. Finally, for comparative purposes, the Brody–Bertalanffy model was assumed and parameters estimated using the program developed by Fabens (1965). Table 3 provides a summary of the results. Method I provides the smallest SSE but based on the likelihood ratio, $R(\theta)$, Methods I and II really are indistinguishable from each other or from the logistic. The likelihood ratio for results from the SAS program is only 0.56. Fabens' program provides a likelihood ratio of only 0.2. The program developed by Causton (Causton et al. 1978) failed to converge. Problems of convergence are discussed by Causton et al. (1978) and D. R. Causton (personal communication) has indicated that Carlander's data would be "difficult" for his program because "the points are mostly near the asymptote ($t = 7$ to 11)." Causton's program requires a large spread of sizes with adequate representation of the very smallest individuals.

TABLE 2. Comparison of Methods I and II with estimates from Causton (1969) based on predicting S_{t+T} from S_t. Parameter b is not included. Data for *Acer pseudoplatanus* (Causton 1969). $N = 11$ pairs. $T = 2$ weeks.

	Causton (1969)	Method II	Method I
n	0.0400	0.0461	2.2480
K	5.2460	4.8876	0.1626
S_∞	21.6976 g	22.5536 g	25.4327 g
m	2.7758×10^{-5}	5.6850×10^{-5}	0.7224
C	3.6782×10^{-34}	4.2784×10^{-30}	0.0658
SSE	18.8276	19.6890	65.7882
$L(\theta)/c$	9.7416×10^{-8}	7.6168×10^{-8}	1.0004×10^{-10}
$R(\theta)$	1.0	0.782	0.001

TABLE 3. Comparison of methods for estimating parameters in the Richards function (excluding the scaling parameter b). Data are for ciscoes (*Coregonus artedii*) from Vermilion Lake, Minnesota (Carlander 1950). SAS is the Statistical Analysis System nonlinear least squares program using Marquardt's algorithm; m and C are slope and intercept in eq (7) and eq (8).

	Method I	Method II	SAS	Logistic (from Method I)	Fabens (1965)	Causton et al. (1978)
n	0.975	0.936	−24.611	+1.0	−1.0	Would not converge
K	0.535	0.536	0.443	0.523	0.341	
S_∞	305.800 mm	305.633 mm	307.772 mm	305.968 mm	312.851 mm	
m	0.586	0.585	0.642	0.587	0.711	
C	2.818×10^{-3}	2.212×10^{-3}	1.162×10^{-3}	1.347×10^{-3}	90.416	
SSE	54.316	54.548	61.789	54.321	77.762	
$L(\theta)/c$	1.559×10^{-9}	1.529×10^{-9}	8.728×10^{-8}	1.558×10^{-8}	3.101×10^{-9}	
$R(\theta)$	1.0	0.981	0.560	0.9996	0.203	

Discussion

The two methods that have been presented appear to be adequate for estimating parameters in the Richards function for many sets of growth data. Determining a priori which method will give the better fit has not been examined since most data sets of interest to me have been sea urchin data as in the first example. Because of the results with Carlander's cisco data it may be that the changeover occurs at about $n = +1$. I have written a computer program that tries both methods with each data set so I have found no need to make an a priori decision. It is also possible that which method provides the better fit is determined solely by the idiosyncrasies of the data such as relative numbers of points near maximum vs. smallest sizes or whether aberrant data points are from small or large individuals.

The methods proposed here, which are derived from the Walford plot, provide estimates of parameters in the Richards function that are about as good or in some cases better than existing techniques for estimating parameters in this function. My searching technique also appears to be less likely to fail at converging than Causton's program and the single example that was tried with the SAS package indicates that the parameters from my methods provide a somewhat better estimate of S_{t+1} from S_t than parameters from the nonlinear least squares method using Marquardt's algorithm.

Mention must be made of problems with very large or very small exponents. Raising 30 to the 25th power can exceed the capacity of some computers. Furthermore, transformed values are squared in calculating regressions, so if a machine is limited by 10^{39} then a value of 7 raised to the 25th power could exceed capacity during the calculation of sums of squares. Methods I and II appear to be cases where the definition formulae for sums of squares should be used rather than the usual calculating formulae. To minimize problems it is probably a good approach to transform data so large numbers are not being raised to large exponents during trial and error solution. Judgement must naturally be applied to transformation because very small fractional values are as much of a problem as very large numbers when using exponents.

The Richards function is a useful addition to population studies not only because it frequently produces a better fit to growth data than the logistic or Brody–Bertalanffy but also because it can be readily incorporated into studies of production or in procedures which attempt to estimate mortality rates from a growth function and size distribution (Ebert 1973; Van Sickle 1977a, b). The Richards function obviously also can be used to estimate parameters of population growth in numbers of individuals. The growth constant K is called r (the growth rate per individual), for population growth and maximum individual size S_∞ would be the carrying capacity K of the environment.

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BAYLEY, P. B. 1977. A method for finding the limits of application of the von Bertalanffy growth model and statistical estimates of the parameters. J. Fish. Res. Board Can. 34: 1979–1984.

CARLANDER, K. D. 1950. Handbook of freshwater fishery biology. W. C. Brown Co., Dubuque, Iowa. 281 p.

CAUSTON, D. R. 1969. A computer program for fitting the Richards function. Biometrics 25: 401–409.

CAUSTON, D. R., C. O. ELIAS, AND P. HADLEY. 1978. Biometrical studies of plant growth. I. The Richards function, and its application in analyzing the effects of temperature on leaf growth. Plant Cell Environ. 1: 163–184.

DAVIS, O. L., AND J. T. KU. 1977. Re-examination of the fitting of the Richards growth function. Biometrics 33: 546–547.

EBERT, T. A. 1973. Estimating growth and mortality rates from size data. Oecologia (Berlin) 11: 281–298.

FABENS, A. J. 1965. Properties and fitting of the von Bertalanffy growth curve. Growth 29: 265–289.

FLETCHER, R. I. 1975. A general solution for the complete Richards function. Math. Biosci. 27: 349–360.

FORD, E. 1933. An accounting of the herring investigations conducted at Plymouth during the years from 1924–1933. J. Mar. Biol. Assoc. U.K. 19: 305–384.

JOHNSON, D. H., A. B. SARGENT, AND S. H. ALLEN. 1975. Fitting Richards' curve to data of diverse origins. Growth 39: 315–330.

NELDER, J. A. 1961. The fitting of a generalization of the logistic curve. Biometrics 17: 89–110.

PIENAAR, L. V., AND J. A. THOMSON. 1973. Three programs used in population dynamics WVONB — ALOMA — BHYLD (FORTRAN 1130). Fish. Res. Board Can. Tech. Rep. 137: 33 p.

RICHARDS, F. J. 1959. A flexible growth function for empirical use. J. Exp. Bot. 10: 290–300.

RICKER, W. E. 1973. Linear regression in fishery research. J. Fish. Res. Board Can. 30: 409–434.

1975. Computation and interpretation of biological statistics of fish populations. Bull. Fish. Res. Board Can. 191: 382 p.

SMITH, J. M. 1974. Models in ecology. Cambridge University Press, Cambridge, England.

SPROTT, D. A., AND J. G. KALBFLEISCH. 1965. Use of the likelihood function in inference. Psychol. Bull. 64: 15–22.

VAN SICKLE, J. 1977a. Mortality rates from size distributions. The application of a conservation law. Oecologia (Berlin) 27: 311–318.

1977b. Mortality estimates from size distributions: a critique of Smith's model. Limnol. Oceanogr. 22: 774–775.

WALFORD, L. A. 1946. A new graphic method of describing the growth of animals. Biol. Bull. 90: 141–147.

Quantitative Measurements of Euphausiids Using a 120-kHz Sounder and Their in situ Orientation

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SAMEOTO, D. D. 1980. Quantitative measurements of euphausiids using a 120-kHz sounder and their in situ orientation. Can. J. Fish. Aquat. Sci. 37: 693–702.

Biomass and density of euphausiids were estimated using a 120-kHz sounder during 2 y in the Gulf of St. Lawrence. Simultaneously, biological samples were taken with two types of multiple opening and closing nets: MOCNESS-type and BIONESS. Correlations for biomass and density estimates between the acoustic and biological data range from 0.391 to 0.791. Acoustic data showed that a high percentage of euphausiids avoided the MOCNESS-type net during the day. A comparison of the relationship between biological and acoustic data for both years showed that the BIONESS sampler captured euphausiids more efficiently during both day and night. Target strength of the euphausiids estimated from acoustic and biological data showed that it was lower than would be expected if the animals were oriented horizontally. Photographs taken from the BIONESS sampler at depths having acoustic scattering layers showed that the average orientation of euphausiids changed with time, the average orientation being closest to horizontal during daytime. Orientation changed from 27° from the horizontal at 14:00 to 51° during 02:00. The effect of orientation on target strength is discussed.

Key words: euphausiids, acoustics, orientation, biomass, underwater photography, multiple net samplers, zooplankton

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Pendant 2 années, on a estimé la biomasse et la densité des euphausides dans le golfe Saint-Laurent à l'aide d'un écho-sondeur de 120 kHz. Des échantillons biologiques ont été prélevés en même temps au moyen de deux types de filets multiples pouvant s'ouvrir et se fermer: MOCNESS et BIONESS. Les corrélations, quant à la biomasse et estimations de densité, entre données acoustiques et biologiques s'étalent de 0,391 à 0,791. Les données acoustiques indiquent qu'un fort pourcentage d'euphausides évitent le filet de type MOCNESS le jour. Une comparaison de la relation entre les données biologiques et acoustiques pendant ces 2 années démontre que l'échantillonneur BIONESS capture des euphausides plus efficacement, tant de jour que de nuit. La résistance de cible des euphausides estimée à partir de données acoustiques et biologiques démontre qu'elle est inférieure à ce qu'on pourrait anticiper si les animaux étaient orientés horizontalement. Des photographies prises depuis l'échantillonneur BIONESS à des profondeurs où se trouvent des couches de dispersion acoustique indiquent que l'orientation moyenne des euphausides change avec le temps: elle se rapproche le plus de l'horizontale le jour. L'orientation passe de 27° par rapport à l'horizontale à 14:00 à 51° à 02:00. Nous analysons l'effet de l'orientation sur la résistance de cible.

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ZOOPLANKTON ecologists for years have attempted to develop means of locating and measuring the concentrations of zooplankton in situ (Maddox and Kanwisker 1965; Longhurst et al. 1966; Boyd and Johnson 1968; Sameoto et al. 1977; Dauphinee 1977). Acoustics have often been considered the brightest hope for development of such an instrument (Moore 1950; McNaught 1969; Barraclough et al. 1969; Bary and Pieper 1970; Beamish 1971; Sameoto 1976; Greenlaw 1977). All these latter studies demonstrated the value of sonar as means of locating animals and providing information on their vertical and horizontal distribution, but none demonstrated that reliable quantitative biomass estimates could be obtained from sonar. One reason for this was the uncertainty introduced by the biological sampling methods (Moore 1950). Animal avoidance of the nets made quantitative relationships between sonar and biological data questionable.

As a result of the development of new and efficient large multiple opening and closing zooplankton nets (Sameoto et al. 1977, 1980), it has now become feasible to relate sonar backscattering data from sound scattering layers quantitatively with zooplankton populations in these layers. The Gaspé coast of the Gulf of St. Lawrence was chosen to study the accuracy of

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acoustics in measuring the density of euphausiids because the composition and distribution of the zooplankton and euphausiid population relative to acoustic scattering layers was well known (Sameoto and Jaroszynski 1973; Sameoto 1976). The zooplankton population during the spring and early summer months is dominated by adult populations of three species of euphausiids and low densities of copepods. There are few pelagic fish in the region and when present these are vertically separated from the layers of euphausiids and copepods. Earlier studies showed that euphausiids form distinct layers at depths between 100 and 125 m during the day, but migrate towards the surface at night (Sameoto 1976).

One of the most important measurements in acoustic estimates of biomass is animal target strength which is very sensitive to changes in the animal's orientation (Samovol'kin 1974). Therefore the other purpose of this study was to look at the in situ orientation of euphausiids and to determine if it changed from day to night. The effect of such an orientation change on the average target strength of the animals and consequently on measurements of biomass and number was examined.

Methods

Sounder

A Simrad Scientific Sounder model EK 120 R with a frequency of 120 kHz and transmitting sound level of 112 dB/1 microbar at 1 m with accuracy of ±0.5 dB was used throughout the study. The settings were constant at 1 ms pulse length, 48 pulses per minute, a 9° beam width, and a bandwidth of 9.9 kHz. The manufacturer rated the time-varied gain ($20 \log r + 2\alpha r$) from 3 to 100 m, but tests showed that it operated to a depth of 125 m. The transducer was mounted in a Braincon 2-ft V-fin (1 ft = 0.3 048 m) towed from the ship's side away from the ship's wake at a depth of 3 m.

The acoustic echo was recorded for future analysis with both 0 and 20 dB amplification, on a Hewlett Packard 3960 tape recorder which had dynamic range of 48 dB and a bandwidth of 2.5 kHz. At the same time the 20-dB-amplified signal was digitized and recorded on magnetic tape using a Hewlett Packard 2100 computer (Fig. 1). At the end of a 4- to 5-min zooplankton tow, the net was closed and the computer calculated the average squared voltage (V^2) of the sonar pulses during the tow for the water column from the transducer to a maximum depth of 125 m, at 1-m intervals. These values were printed and a V^2 profile with depth plotted. The depth of the layers was corrected for the transducer depth.

Only acoustic data not contaminated by large voltage spikes several times above the intensity of the signal coming from the euphausiid layers were used. It was possible also to detect large sound scatterers such as fish from the traces of the echograms. When these were present in the scattering layers, the data were rejected from this study. Therefore, the correlation involved only echos from layers of objects that gave a uniform intensity echogram. Because of a number of uncertainties such as target strength, animal orientation, and number of animals in the acoustic beam, the approach to signal analysis was kept as simple as possible. The

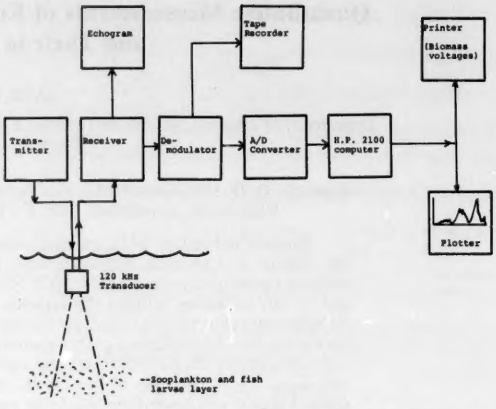

FIG. 1. Block diagram showing sonar and computer arrangement.

components extracted from the acoustic signal and correlated with the net catches were V^2 and volume backscattering (S_v). S_v is defined as the ratio of intensity of sound scattered back in the direction of the source by a unit volume, to the intensity of the incident plane wave:

$$S_v(\text{dB}) = 10 \log_{10} \frac{I_{\text{scattered}}}{I_{\text{incident}}}.$$

The sound intensity at the transducer caused by the scatterers in the insonified volume can be written in terms of the voltage output of the system:

$$I \simeq \left(\frac{V}{VR}\right)^2$$

where V is the root-mean-squared voltage output and VR the total voltage gain of the system including transducer characteristics, time-varied gain, source strength, and system gain. It was a constant for this experiment.

The volume backscattering was calculated using the basic sonar equation as follows:

$$20 \log V = 20 \log G + SL - (20 \log r + 2\alpha r) + S_v + 10 \log \frac{CT}{2} + 10 \log \psi$$

where: V = voltage output, G = amplifier gain (or receiver gain), SL = source level in decibels relative to 1 microbar at 1 m, r = range in metres, ψ = ideal beam pattern which is equivalent to the real beam pattern, α = absorption coefficient in decibels per metre, T = pulse duration, C = sound velocity (both T and C are in seconds).

Volume backscattering can be expressed as $S_v = 20 \log V - K$ where the value of K which is the product of all the constants in the above equation (with the transducer in a 2-ft V-fin and using 100 m of towing cable, a 1-ms pulse length, and a 20-dB gain in the demodulator) was 55.7 dB.

The squared voltage values (V^2) used in the correlations with euphausiid numbers and biomass were corrected for the system noise by subtracting the squared voltage reading from depths at which euphausiids or significant num-

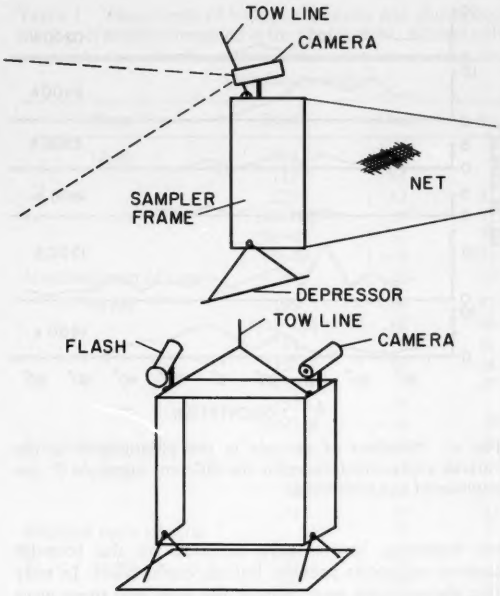

FIG. 2. BIONESS showing position of the camera on the sampler with the field of view shown with broken lines.

bers of other zooplankton did not occur. This was considered to be the noise level of the electronic system and ranged between 0.006 and 0.007 V^2 from day to day and at different locations in the region. Therefore the calculated regression lines for V^2, numbers, and biomass of euphausiids should theoretically pass through the origin when there were no euphausiids or other zooplankton present.

SAMPLING AND PHOTOGRAPHIC METHODS

Biological samples were taken with two types of multiple opening and closing nets. In 1976, the sampler used was that described by Sameoto (1976) and resembled MOCNES (Wiebe et al. 1976). It was able to take 10 separate samples on command. The sampler had sensors for temperature, depth, pitch, and roll, as well as flowmeters in each net that started counting when the net opened, and stopped when it closed. A new improved multiple opening and closing net sampler, called BIONESS (Sameoto et al. 1980), was used in 1977. It was designed to minimize the pressure wave in front of the net. In addition to the 10 nets and the sensors mentioned above, BIONESS had on it a conductivity cell and an EG and G International Inc. Edgerton model 205 camera with a f/4.5 lens and a model 206 light source. The film used was Kodac Plus-X black and white. The camera and light source were mounted on top of the frame on the BIONESS sampler (Fig. 2). The camera was mounted to point downward in front of the sampler at an angle of 10° and gave a field of view of 51° horizontally and 33° vertically. It was focused at a distance of 2 m in front of the net which gave a field width at this distance of about 1.9 m and a height of 1.2 m.

At the same time the net samples were taken, the camera was triggered from the deck of the ship with the result that the depth and exact time of each photograph was known. The photographs were compared to the biological catches and the sonar record at the depth at which the photographs were taken. A total of 350 photographs were taken at different depths in and out of acoustic scattering layers.

The shape of the euphausiids was easily recognizable from the photographs although species identification was impossible. In interpreting the photographic data only objects that could confidently be identified as euphausiids were included. When measuring the angle of the euphausiids, only animals that were broadside to the camera were used, since any other position would not give a true indication of the orientation of the animal.

The animal's angle was measured from the horizontal and assigned an angle from 0° to 90°. Since the important factor was whether the head was pointing up or down and not whether to the right or left, the angles above the horizontal line were given positive values and those below the line negative values. The 10° tilt of the camera distorted the position of the animals by less than 1° and therefore was ignored in the measurements. Both samplers incorporated in their design a depressor which made them extremely stable at all depths and very easy to maintain in the scattering layer of interest.

The sampling procedure, in both years, was to lower the sampler to the depth of interest and tow it at 3 knots. Individual nets were opened and closed at different depths at the same time as the sonar echos were recorded. Samples were taken at depths where sonar scattering occurred as well as above and below scattering layers, and also in areas where there was no sign of sound scattering at any depth. Sampling occurred over a 10-d period during the day and night in both years.

The tows were usually of 4- to 5-min duration for each individual net during which time about 300 m^3 of water was filtered. Closure of each net was recorded on the echogram and tape recorder. The mean numbers of animals taken, and their weights per cubic metre filtered, were later correlated with the mean V^2 per cubic metre at that depth during the tow.

All the biomass values are wet weights from a solution of 5% formalin in seawater.

Nets of two mesh sizes, 243 and 1000 μm, were used on the samplers. A '*t*' test showed no significant difference in the number of euphausiids caught between both mesh sizes. The advantages of using the finer mesh was that it also caught the small copepods present with the euphausiids. During 1976, a total of 90 biological samples, and in 1977, 60 samples, were taken simultaneously with the acoustic data.

Results

SPECIES COMPOSITION

The population of macrozooplankton in the study area consisted entirely of three species of euphausiids. In 1976 *Meganyciphanes norvegica* was dominant making up 85% of the population of euphausiids, while *Thysanoessa raschii* made up 11% and *T. inermis* 4% of the remaining population. Copepods were also

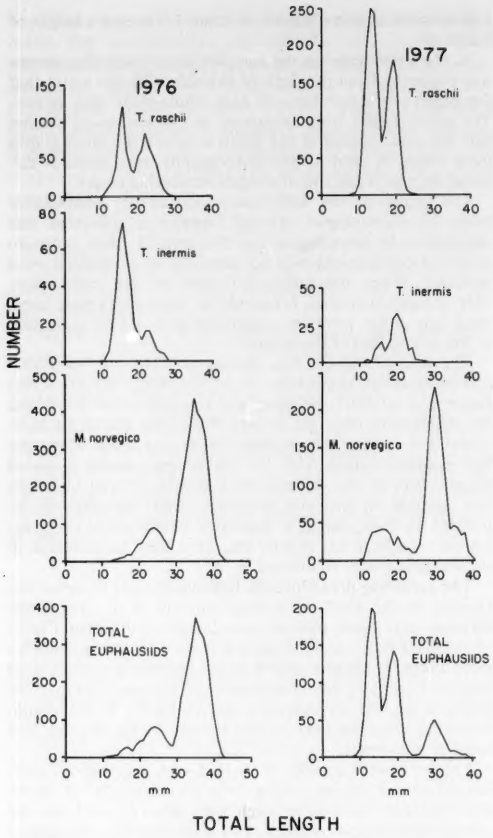

FIG. 3. Length–frequency relationship for different species of euphausiids and the total population during 1976 and 1977.

present, dominated by *Calanus finmarchicus*, *C. hyperboreous*, and *Pseudocalanus elongatus*. *Sagitta* spp. were present in very low numbers.

The macrozooplankton population in 1977 differed from 1976 in the dominant species. *Thysanoessa raschii* made up 77%, *M. norvegica* 28%, and *T. inermis* about 2% of the population. The difference in species ratios in both years meant that the average body size of euphausiids in both years was different. The average body length was larger in 1976 (30.2 ± 3.7) than in 1977 (18.5 ± 3.0) (Fig. 3).

1977 Photographic Measurements of Orientation

The photographs taken in the scattering layers showed copepods, chaetognaths, and large numbers of euphausiids present. Photographs taken at depths where

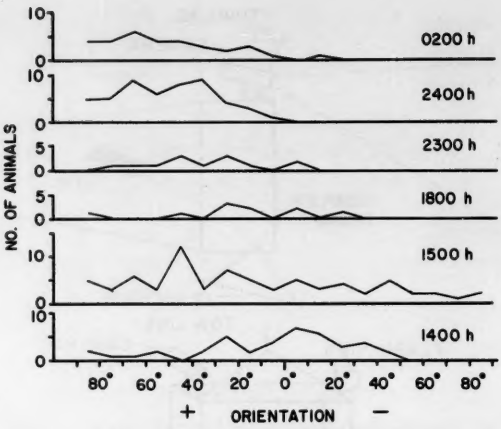

FIG. 4. Numbers of animals in the photographs at the various angle orientations for the different times. At 0° the euphausiid was horizontal.

no scattering layers were detected by the sounder showed copepods present, but no euphausiids. In only two photographs were pelagic fish seen and these were taken during the day at a depth of 40 m in a very strong acoustic scattering layer much more intense than layers caused by euphausiids. Fish were never photographed in the deeper layers where euphausiids were seen and captured. The net samples did not contain any types of animals not photographed.

The mean orientation of the euphausiids changed progressively from mid-afternoon to night and early morning (Fig. 4 and Table 1). At 14:00 and 15:00 there was almost a uniform distribution of orientation angles from −90 to +90. But as the day progressed there were fewer negative angles and more animals with larger positive angles. There was no obvious difference between the angle of orientation and the different species. This was determined by comparing the samples that were dominated by either *M. norvegica* or *T. raschii* (Table 1).

1976 Acoustic Data

When the sonar echo voltages (V^2) were plotted against biomass and numbers of euphausiids (Fig. 5), the slopes were different for day and night (Table 2). The backscattering voltage was higher for a given biomass and number during the day with the ratio between the slopes for day and night regressions being 5.6 and 7.0 for biomass and numbers, respectively.

No other large species of zooplankton were caught and there was no evidence of large single targets such as fish. The fact that the samples were taken in the same region during day and night during a 10-d period during which there was no significant difference in

TABLE 1. Time, depth of biological samples and photographs, the mean and standard deviation of the orientation angle, and number of animals measured in the photographs. All data collected during one cruise in April 1977.

		Orientation angle			% species		
Time	Depth	Mean	SD	*n*	*M. norvegica*	*T. raschii*	*T. inermis*
14:00	115	86		1	34	65	1
	117	84		1	30	69	1
	125	52	37	2	78	19	3
	127–100	23	32	6	79	19	2
	98–60	37	32	2	49	51	3
	60–40	−8	22	29	7	88	9
Absolute mean of total		27	22	41			
15:00	155	10	53	25	100		
	155–164	18	43	16	100		
	154	0.8	83	2	100		
	155	28	36	16	100		
	155	17	47	12	100		
	157	18		1	100		
	156–138	−19	57	3	90	10	
Absolute mean of total		42	25	75			
18:00	95	52		1	1	99	
	98	−69		1	1	98	1
	93	14	44	12	2	98	
Absolute mean of total		37	27	14			
23:00	97	30		1	44	54	2
	100	44	22	6	52	44	4
	125	27	25	6	86	12	2
Absolute mean of total		36	22	13			
24:00	100	37	38	13	81	19	
	100	79	8	2	73	27	
	100	39	25	5	79	21	
	100	54	19	6	84	15	1
	125	47	18	12	84	16	
	125	28	46	10	92	8	
	125	63	19	6	99	1	
Absolute mean of total		50	20	54			
02:00	23–40	33	45	8	3	94	3
	90	43	28	6	34	64	2
	148	62	22	8	93	7	145
	148–125	20	53	2	85	14	1
	125	54	17	9	84	14	2
Absolute mean of total		51	23	33			

species composition meant that the same zooplankton population was being sampled.

During the day, sonar scattering layers were much more intense because the euphausiids concentrated at a depth between 100 and 125 m. Plankton tows in water above this layer caught no euphausiids and samples below it had very few animals in them. At dusk the layer migrated toward the surface and spread over a wider depth range (Sameoto 1976). Two regression equations were calculated for the V^2 and biological data, one that passed through the origin and the other with an intercept determined by the data (Table 2). There were no significant differences between the slopes of these lines which indicated that the assumption that the backscattering was due entirely to euphausiids was probably correct.

1977 ACOUSTIC DATA

In 1977 data were treated in the same manner as in 1976 (Fig. 6). The regression lines for daytime tows and V^2 were different from night regression lines, with the voltages from the backscattering higher for a given biomass and number than during the night. The difference ratio between the slopes of these lines was less, 1.42 and 1.80, than it was in 1976.

There was no significant difference between the body lengths of animals in any of the samples used in the analysis in 1976. In 1977 two samples taken at night had larger animals than the rest of the night or day samples. The mean body length for these was 24 mm against 16 mm in the other samples. This larger body length may be the reason these two samples gave rela-

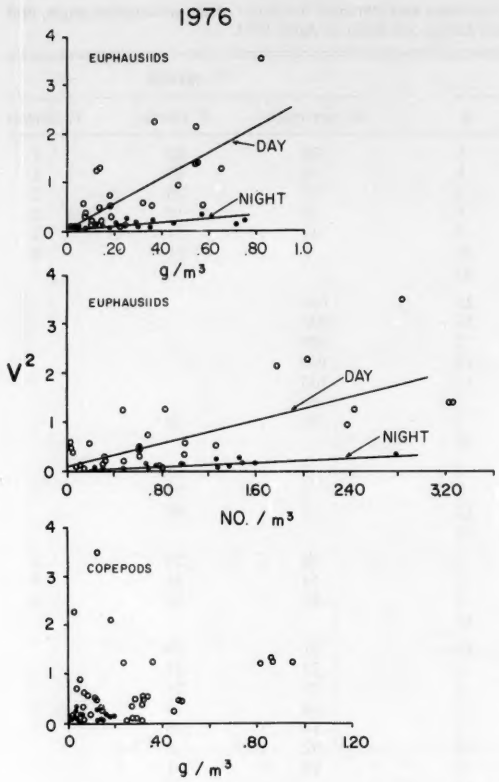

FIG. 5. Regression lines between squared backscattering voltage (V^2) and euphausiid numbers and biomass and copepod biomass for 1976. (●), nighttime data; (○), daytime data.

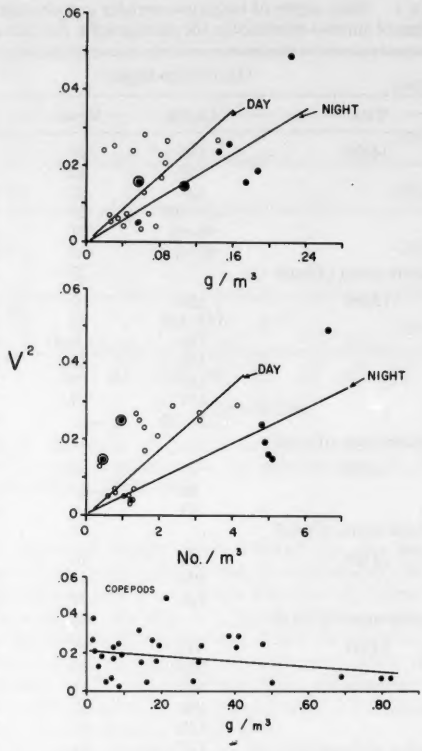

FIG. 6. Regression lines between squared backscattering voltage (V^2) and numbers and biomass of euphausiids and copepod biomass for 1977. (●), nighttime data, (⊙), nighttime data with large animals, (○), daytime data.

tive high voltage readings for the numbers per cubic metre data (Fig. 6), but this is uncertain because of the small number of samples.

Biomass values were lower in 1977 than in 1976 with the result that V^2 values were much lower. A statistical comparison of those 1976 data which fell within the range of the 1977 data showed that both the means and the slopes were significantly different ($F = 38.39$, $df = 1, 28$ for homogeneity of the slopes and $F = 38.39$, $df = 1, 28$ for the means). There was no significant difference between the correlation coefficients for the regression equations (Table 2) between the day and night data in each of the years. This means that the amount of variation for the samples and V^2 was not different between day and night. The correlation coefficients were lower during 1977 than in 1976. The low correlation coefficient value for daylight biomass may be due to the narrow range of biomass values caught, 0 to 0.08 $g \cdot m^{-3}$, causing the variance to mask the regression with the V^2. As a result, when the data were forced through the origin the difference between the data slope and forced slope was much larger than for any of the other data pairs (Table 2).

The Effect of Copepods on V^2

The relationship between V^2 and copepod biomass was determined by comparing samples in which there were only copepods and samples that had both euphausiids and copepods. The 1976 data was divided into day and night samples, while the 1977 data were combined. There was no significant correlation between biomass of copepods and V^2 in either year (Fig. 5 and 6). The maximum number of copepods in 1976 was $311 \cdot m^{-3}$ and $290 \cdot {}^{-3}$ in 1977. When only copepods were present in the samples, no scattering layers were observed.

TABLE 2. Regression equations between $V^2(y)$ and the biological components (x) of biomass ($g \cdot m^{-3}$) and numbers m^{-3}.

Biological component	Regression equation	Coefficient of correlation	No. of samples	Standard error of estimate	Regression equation when forced through the origin
	1976 daylight				
Euphausiid biomass	$y = 0.0588+2.70\,x$	0.765	33	0.527	$y = 2.80\,x$
Euphausiid numbers	$y = 0.241+0.67\,x$	0.764	33	0.500	$y = 0.70\,x$
Copepod biomass	$y = 0.4462+0.77\,x$	Not sig.	40		
	1976 night				
Euphausiid biomass	$y = -0.0276+0.43\,x$	0.697	16	0.065	$y = 0.50\,x$
Euphausiid numbers	$y = 0.0092+0.12\,x$	0.791	16	0.055	$y = 0.10\,x$
Copepod biomass	$y = 0.1339+0.03\,x$	Not sig.	19		
	1977 daylight				
Euphausiid biomass	$y = 0.01+0.070\,x$	0.210	20	0.09	$y = 0.22\,x$
Euphausiid numbers	$y = 0.002+0.008\,x$	0.729	20	0.006	$y = 0.009\,x$
Copepod biomass (day and night)	$y = 0.022-0.010\,x$	−0.374	30		
	1977 night				
Euphausiid biomass	$y = 0.001+0.146\,x$	0.668	7	0.008	$y = 0.155\,x$
Euphausiid numbers	$y = 0.015+0.002\,x$	0.392	7	0.010	$y = 0.005\,x$

V^2 AND VERTICAL MIGRATION

The vertical migration of euphausiids at dusk could be followed with the sounder. A region was selected for study that had a uniform scattering layer over a large area. There was no pronounced thermocline at this time but rather a three-layered water system which was common in the region. The euphausiids were usually found at a depth just above the warmer deep layer (Fig. 7) during the day. The average total V^2 over the depth of the scattering layer was determined at 3- to 4-min intervals as zooplankton samples were taken in the layer. The average V^2 of the entire layer changed little as the layer migrated upward and spread over a wider depth range (Fig. 7, Table 3). This indicated the animals likely did not interact to affect the strength of the scattering voltage of the layer as their density changed and therefore the scattering was incoherent at all the observed densities. It was possible to estimate the total biomass of euphausiids in the scattering layer by extrapolating the daytime V^2, biomass regression to the voltages recorded. It was found that the biomass ranged between 9.2 and 11.9 $g \cdot m^{-2}$ (Table 3).

The V^2 spikes between 0 and 50 m were likely small schools of fish (Fig. 7). The large V^2 in the top 10 m was probably the result of air bubbles trapped in the water due to wave action. Samples and photographs taken in this layer did not show any concentration of zooplankton or fish.

TABLE 3. Average sum of V^2 in scattering layer recorded April 18, 1977, and the calculated total biomass of eupahusiids $g \cdot m^2$ in the layer.

Depth range of layer	Time (EDT)	V^2	Estimated total biomass, $g \cdot m^{-2}$
106–124	1903	2.23	10.1
100–124	1907	2.61	11.9
100–120	1911	2.26	10.3
90–120	1915	2.49	11.3
80–120	1920	2.56	11.6
80–120	1923	2.53	11.5
70–120	1927	2.02	9.2
70–120	1930	2.48	11.3

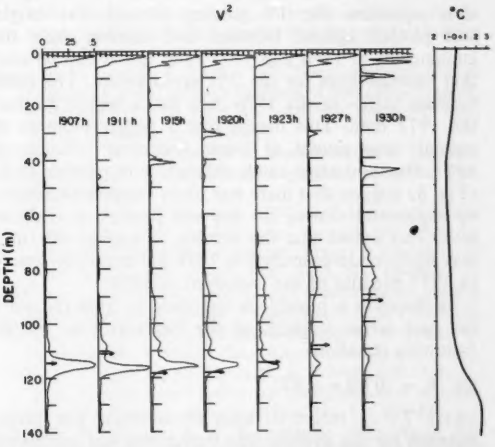

FIG. 7. Profile of backscattering voltage squared with depth and time. Arrows indicate depth at which plankton tows were made. Temperature profile as the average during the tow.

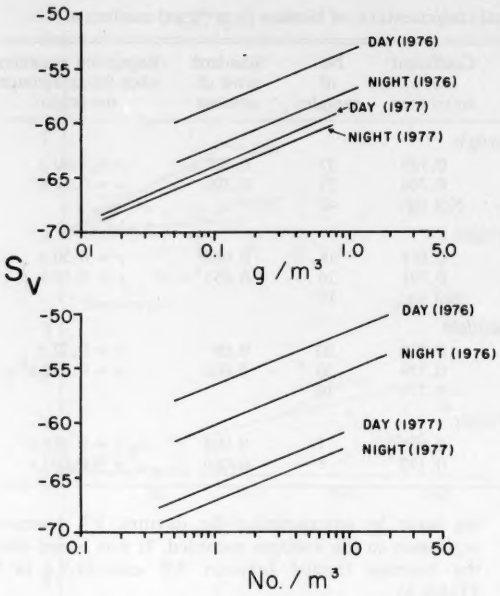

FIG. 8. Relationship between biomass and numbers of euphausiids and volume backscattering (S_v) for day and night data during 1976 and 1977.

RELATIONSHIP BETWEEN VOLUME SCATTERING, BIOMASS, AND NUMBERS

Volume backscattering S_v was calculated from regression equations for V^2 passing through the origin and plotted against biomass and number data for euphausiids in 1976 and 1977 (Fig. 8). It can be seen that biomass lines for the 2 yr are different. The same biomass values in the 1976 data had a higher S_v than the 1977 data. This means that a higher biomass of animals was caught at lower scattering volumes in 1977. Day and night catch differences in relation to S_v (Fig. 8) suggest that there was likely sampler avoidance by euphausiids during the day and possibly at night as well. This means that the number of euphausiids $\cdot m^{-3}$ was likely underestimated in 1976 but probably less so in 1977 because of the improved sampler.

In theory it is possible to calculate S_v from the number and target strength of the backscatterers by the following equation:

$$(1)\quad S_v = 10 \log n + TS$$

where TS = target strength in decibels. The target strength for the average size euphausiid was calculated from its geometrical cross section and assuming that its acoustic reflectivity was 5%. The value of 5% was estimated from the work on shrimp by Samovol'kin (1974). It was estimated that a 35-mm long *M. norvegica* in a horizontal position had a TS of −65 dB. This was the dominant species in 1976. The S_v values calculated from V^2 and S_v calculated from n and a TS of −65 dB were very similar, but unfortunately could not be correct. Since the day–night catch comparison suggested that the 1976 sampler did not catch all the animals present, values of n were too low. In addition, photographic evidence showed the average orientation to be between 27° and 42° from the horizontal during the daytime and up to 51° during the nighttime. This meant that the average TS at all times was less than the estimated −65 dB TS of an animal in the horizontal position (Samovol'kin 1974; Greenlaw 1977). Therefore, there were two unknowns in eq (1) for S_v. It was possible to estimate the correct value of n by comparing the catches for 1976 and 1977 and making the assumption that the 1977 catches were 100% efficient. By extrapolating the line for the 1977 data to the highest V^2 and therefore S_v recorded in 1976, a biomass of 3.13 g $\cdot m^{-3}$ was obtained at a S_v equal to −62 dB. The average wet weight of the euphausiids in 1976 was 0.28 g which meant that there were about 11.2 euphausiids $\cdot m^{-3}$ or about 5.2 times as many animals as were caught. Therefore, to obtain an S_v of −62 dB with a density of 11.2 animals $\cdot m^{-3}$ using eq (1), the TS of the average animal would have to be −73 dB, which is considerably less than the estimated −65 dB TS for a euphausiid of this size in the horizontal aspect. Greenlaw (C. F. Greenlaw personal communication) estimated the TS of a 35-mm live euphausiid in the horizontal position to be ~ −77 dB. If this is correct, then the difference between the acoustic estimates and the actual catches in 1976 would be much greater and in the case of S_v equal to 62 dB, there would have to be 31 euphausiids $\cdot m^{-3}$. This estimate of the number of euphausiids does not take into account the deviation of over 27° from the horizontal orientation of the animals (Table 1) which would reduce the scattering strength of the euphausiids, which means there were probably more than 31 euphausiids $\cdot m^{-3}$.

Using the same reasoning with the 1977 data, i.e. that the net was 100% efficient, it was calculated that the TS of the animals had to be about −80 dB to give the measured S_v values. The mean length of the population of euphausiids was 18.5 mm, which was much lower than in 1976 (Fig. 3). Greenlaw (1977) found the TS at 100 kHz of preserved euphausiids, 23-mm length, to range between −78 and about −73 in the horizontal orientation. Therefore, an average TS of −80 dB for animals caught in 1977 is not unreasonable considering the TS reduction due to the animals orientation.

Discussion

The primary purpose of this study was to test the accuracy of acoustics in providing quantitative data on an in situ population of animals that could be easily caught and used to verify the estimates obtained from

the sounder using laboratory estimates of *TS*. The euphausiids were chosen as the animals on which to run these experiments because of their known distribution and relatively uniform size and species composition which reduced the acoustic signal variability. It was felt that with the high quality of electronic and sampling equipment at our disposal, that the results obtained would be the most accurate to date dealing with this problem. This study demonstrated that the 120-kHz sounder was a powerful tool in locating concentrations of euphausiids and providing information on the vertical and horizontal distribution of the animals. Its use as a quantitative instrument was also demonstrated but with a greater degree of uncertainty than the qualitative information.

One of the most important elements in determining the number or biomass of euphausiids with the use of acoustics is the *TS* of the animals. Unfortunately this is the component of the acoustic equation that has the most uncertainty about it. The laboratory measurements of *TS* of euphausiids of different lengths have been done on preserved animals and in freshwater (Greenlaw 1977). Both factors may give larger *TS* values than the in situ. The other uncertainty comes from the orientation of the animals.

Euphausiids are highly directional acoustic scatterers (Greenlaw 1977) with changes in the target strength as great as 30 dB occurring as the animal turns from a horizontal to a vertical position. No information on the target strength of euphausiids in the vertical position with the head pointing down is available, but a study by Samovol'kin (1974) with preserved crabs showed very little difference in the target strength between the animal in a head up or head down position. If, as Samovol'kin's results suggested, it does not matter if the animal is pointing up or down, but only the degree it deviates from the horizontal position that effects the target strength, then the changes from the mean day position to the night position would be about 20°. Unfortunately there are no data on the amount of target strength reduction caused by a change in orientation of 20°, the works of Samovol'kin and Greenlaw refer only to dorsal and anterior positions. If the assumption is made that the change of −30 dB in target strength is linear with the orientation angle from 0° to 90°, then a change of 20° would result in a drop of 7 dB in target strength which could cause an underestimation of the population by about a factor of 5.

Laboratory or theoretical estimates of target strength of aquatic animals are usually based on the assumption that the animal is in a horizontal position with its dorsal side pointing up. But as this study showed, there were very few animals at any time in this position. This means that the average target strength for a population of euphausiids will always be lower than the calculated horizontal position. Other sources of error exist in the interpretation of acoustic data. When processing the signals it is assumed that the target distribution is such as to produce an incoherent backscattering signal. But even if a small percentage of the scatterers are coherent, the density estimate errors could be rather large. Fortunately, backscattering data on the vertically migrating layer of euphausiids suggested that the most probable distribution of animals at the low densities found in 1977 was one that produced incoherent signals. When all the possible sources of error in estimating the biomass and numbers of euphausiids from V^2 are considered, the amount of variance in the data about the regression lines was surprisingly small.

The acoustic echo from a layer of zooplankton depends on the density·m^{-3} of the animals and the size of the animals relative to the frequency of the sounder. Therefore, the same biomass of copepods and euphausiids would not produce the same backscattering voltage. The 120-kHz sounder and electronic system used was a very effective tool in locating euphausiids provided their density was at least 1 m^{-3} and biomass 0.04 $g \cdot m^{-3}$, but it did not record concentrations of copepods in densities up to 1.0 $g \cdot m^{-3}$ wet weight. This sounder was well suited for quantitative studies of euphausiids in this region since when the euphausiids were present the densities and numbers usually exceeded these limiting values (Sameoto 1976).

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BARRACLOUGH, W. E., R. J. LEBRASSEUR, AND O. D. KENNEDY. 1969. Shallow scattering layer in the subarctic Pacific Ocean: detection by high-frequency echo sounder. Science 166: 611–613.

BARY, B. M., AND R. E. PIEPER. 1970. Sonic-scattering studies in Saanich Inlet, British Columbia: a preliminary report, p. 601–611. *In* G. B. Farquhar [ed.] Proc. Int. Symp. Biol. Sound Scattering in the Ocean.

BEAMISH, P. 1971. Quantitative measurements of acoustic scattering from zooplankton organisms. Deep-Sea Res. 18: 811–822.

BOYD, C., AND G. W. JOHNSON. 1968. Studying zooplankton populations with an electronic zooplankton counting device and the LINC computer. Trans. Application of Seagoing Computer Symp. Mar. Tech. Soc. 83–90 p.

DAUPHINEE, T. M. 1977. Zooplankton measurements using a conductance cell. Oceans '77 Conf. Rec. 2: 39B-1–39B-5 p.

GREENLAW, C. F. 1977. Backscattering spectra of preserved zooplankton. J. Acoust. Soc. Am. 62: 44–52.

LONGHURST, A. R., A. D. REIGH, R. E. BOWER, AND D. L. R. SEIBERT. 1966. A new system for the collection of multiple serial plankton samples. Deep-Sea Res. 13: 213–222.

MADDOX, W. S., AND J. W. KANWISHER. 1965. An *in situ* particle counter. Limnol. Oceanogr. 10(Suppl.): R162–168.

MCNAUGHT, D. C. 1969. Developments in acoustic plankton sampling. Proc. 12th Conf. Great Lakes Res. 61–68 p.

MOORE, H. B. 1950. The relationship between the scattering

layer and the Euphausiacea. Biol. Bull. (Woods Hole, Mass.) 99: 181–212 p.

SAMEOTO, D. D. 1976. Distribution of sound scattering layers caused by euphausiids and their relationship to chlorophyll *a* concentrations in the Gulf of St. Lawrence estuary. J. Fish. Res. Board Can. 33: 681–687.

SAMEOTO, D. D., AND L. O. JAROSZYNSKI. 1973. Distribution of euphausiid scattering layers in the Gulf of St. Lawrence estuary. Fish. Res. Board Can. Tech. Rep. 430: 17 p.

SAMEOTO, D. D., L. O. JAROSZYNSKI, AND W. B. FRASER. 1977. A multiple opening and closing plankton sampler based on the MOCNESS AND N.I.O. nets. J. Fish. Res. Board Can. 34: 1230–1235.

1980. BIONESS, a new design in multiple net zooplankton samplers. Can. J. Fish. Aquat. Sci. 37: 722–724.

SAMOVOL'KIN, V. G. 1974. Acoustic backscattering cross sections of small fish, crustaceans and medusae as a function of their fore shortening. Okeanologiya 14: 655–660.

WIEBE, P. H., K. H. GURT, S. H. BOYD, AND A. W. MORTON. 1976. A multiple opening/closing net and environmental sensing system for sampling zooplankton. J. Mar. Res. 34: 313–326.

La mesure du pH en milieu estuarien sur l'échelle NBS

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PELLETIER, E. ET J. LEBEL. 1980. La mesure du pH en milieu estuarien sur l'échelle NBS. Can. J. Fish. Aquat. Sci. 37: 703–706.

Cet article propose une adaptation de la méthode classique de mesure du pH sur l'échelle NBS (National Bureau of Standards) pour fin d'utilisation en milieu estuarien et côtier. La fidélité est de ±0.005 unité de pH pour 95% des échantillons estuariens analysés. L'utilisation d'un étalon secondaire à force ionique élevée permet de suivre et de corriger la dérive lente des électrodes se produisant pendant une longue série d'analyses en mer. La précision est estimée à ±0.010 unité de pH et la méthode ne semble pas sensible aux variations brusques de la force ionique entre des échantillons successifs. L'application de cette méthode simple et pratique peut contribuer à augmenter notre connaissance de la variabilité du pH en milieu estuarien.

Mots clefs: pH, estuaire, variation, force ionique

PELLETIER, E. ET J. LEBEL. 1980. La mesure du pH en milieu estuarien sur l'échelle NBS. Can. J. Fish. Aquat. Sci. 37: 703–706.

An adjustment is proposed to the classical method of measuring pH in an estuarine and inshore environment. The reproducibility obtained is ±0.005 pH units for 95% of analyzed samples. The use of a secondary standard with high ionic strength makes it possible to follow and correct the slow drift of electrodes which usually happens when a long series of analyses is made on board. The method is estimated to be accurate to ±0.010 pH units and does not appear to be sensitive to abrupt variations of ionic strength of successive samples. The use of this simple and practical method can contribute to a better knowledge of pH variability in estuaries.

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DEPUIS quelques années, les échelles utilisées pour la mesure du pH en milieu marin ainsi que l'interprétation des résultats obtenus par différentes méthods ont soulevé plusieurs controverses. D'un côté, le concept classique utilisant les tampons NBS (National Bureau of Standards) a été principalement défendu par Pytkowicz et al. (1966) et Johnson et al. (1977). D'autre part, un nouveau concept utilisant des tampons à force ionique élevée a d'abord été proposé par Hansson (1972) et ensuite appliqué à l'eau de mer par Almgren et al. (1975). Une revue critique de ces méthodes a été faite par Bates (1975). Malheureusement le milieu estuarien n'a pas été considéré dans ce débat de fond et peu de développement pratique en a résulté pour les chercheurs s'intéressant aux eaux saumâtres des estuaires et des zones côtières.

Le but de ce travail est d'abord de poser le problème particulier rencontré dans les zones de mélanges estuariennes, de proposer une méthode simple et pratique de la mesure du pH en mer et d'estimer la précision de cette méthode.

Méthodologie — Du point de vue analytique, le caractère essentiel du milieu estuarien est l'extrême variabilité de la force ionique. Comme le pH est avant tout une mesure potentiométrique, cette variation du milieu ionique où sont plongées les électrodes entraîne inévitablement une instabilité de la lecture et une incertitude sur la justesse et la fidélité de la réponse obtenue. Pour solutionner ce problème, Almgren et al. (1975) ont proposé un réétalonnage de la paire d'électrodes avec un tampon de force ionique appropriée dans le cas où une variation de plus de 10% de la salinité apparaît entre deux échantillons successifs. Une telle méthode est impraticable au cours d'une mission océanographique en milieu estuarien car elle suppose:

1) l'utilisation d'une dizaine de tampons différents pour couvrir toute la gamme de salinité de 0 à 35‰,
2) la connaissance immédiate et précise de la salinité de l'échantillon à analyser,
3) un temps considérable consacré aux nombreux réétalonnages en plus de l'accumulation des risques d'erreurs de manipulation.

Ainsi, la méthode de Almgren et al. (1975) ne pouvant être retenue, nous avons opté pour la méthode classique

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utilisant d'échelle NBS (Pytkowicz et al. 1966) et fournissant une mesure du "pH apparent" tel que définie par Park (1968). Johnson et al. (1977) ont montré que la précision de cette méthode en milieu océanique était de ±0.010 pH à un degré de confiance de 95%. Nous avons cherché à obtenir le même ordre de précision en adaptant cette méthode au milieu estuarien.

Au laboratoire, nous avons observé un ajustement rapide de la réponse à pH ≃8 au moment du transfert des électrodes du tampon NBS (phosphates 0.025 mol/L, pH 6.865 à 25°C) à un échantillon d'eau de mer standard (S:35‰ en vase clos à 25°). Ce premier mouvement précède une dérive de plus en plus lente dans les heures suivantes. Cette dérive est irréversible et nous indique l'adaptation ionique plus ou moins rapide et peu reproductible en fonction du temps des électrodes à leur nouveau milieu ionique. Cette simple expérience nous indique une dérive à court terme mais ne nous renseigne pas sur la possibilité d'une dérive à long terme pouvant se produire au cours d'une mission océanographique de plusieurs jours. Afin de suivre toute dérive lente ou modifications brusques de la réponse des électrodes sans devoir refaire l'étalonnage, nous proposons l'utilisation d'un tampon à force ionique élevée pouvant servir d'étalon secondaire pendant toute la durée de la mission océanographique.

Étalonnage — Au moins 24 h avant le début de la mission en mer, la paire d'électrodes verre et calomel (Radiometer® G2040C et K4040) est soigneusement étalonnée avec deux tampons NBS (phosphates pH 6.825 et phthalate pH 4.008 à 25.0°C). Aucun réétalonnage ultérieur ne sera effectué pendant la mission tel que le recommande Zirino (1975). Les électrodes sont plongées dans une solution de tampon TRIS (2-amino-2-hydroxymethyl propane-1,3-diol) d'une salinité approximative de 18‰ préparé à la manière d'Almgren et al. (1975). Le tampon TRIS sert à maintenir les électrodes dans un milieu ionique constant semblable à l'eau estuarienne et sera utilisé ensuite comme étalon secondaire pour détecter et suivre toute dérive à long terme des électrodes pendant la mission. L'adaptation des électrodes au milieu ionique fort est lente et un équilibre apparent est obtenu après 10 à 12 h d'immersion dans le tampon TRIS sous une agitation continue. Au moment de commencer la série de mesures en mer, le pH de l'étalon secondaire (tampon TRIS) est noté soigneusement pour vérification ultérieure.

Échantillonnage et mesures — La procédure d'échantillonnage est identique à celle utilisée pour l'oxygène dissous (Carritt et Carpenter 1966). Les bouteilles d'échantillons (polypropylène, 125 cm^3) sont fermées de façon à n'emprisonner aucune bulle d'air à l'intérieur et déposées dans un bain thermostaté à 25.0°C pour environ 1 h. Les mesures simultanées du pH et de la température sont ensuite effectuées directement dans la bouteille d'échantillon afin d'éviter tout échange gazeux avec l'atmosphère. Les électrodes et une sonde à température sont ajustées fermement au goulot de la bouteille d'échantillon à l'aide d'un bouchon de caoutchouc recouvert d'une couche de Teflon®; l'excès d'eau coule à l'extérieur et aucune bulle d'air ne doit être retenue à l'intérieur de la bouteille. Avec une agitation adéquate, l'équilibre entre les électrodes et l'échantillon est établi après environ 3 min. Pendant cette courte période de temps, la variation de température ne doit jamais excéder ±0.1°C. Tous les échantillons d'une même palanquée sont successivement mesurés et le couple d'électrodes est ensuite plongé dans une solution du tampon TRIS en attendant une nouvelle série d'échantillons. Il suffit d'essuyer délicatement les électrodes entre chaque analyse car elles ne doivent *jamais* être lavées à l'eau distillée. A toutes les deux heures, le pH d'une solution fraîche de tampon TRIS à 25.0°C est mesuré selon le procédé décrit ci-haut et noté soigneusement en fonction du temps afin d'établir la dérive possible des électrodes.

La mesure d'échantillons de très faibles salinités (S <5‰) peut causer certaines difficultés. La réponse des électrodes bien adaptées aux fortes salinités est de plus en plus instable à mesure que l'on s'approche de l'eau douce. En doublant le temps de contact entre les électrodes et l'échantillon, on peut arriver à stabiliser cette réponse.

Résultats et discussion — Fidélité de la méthode — Nous avons expérimenté cette méthode au cours d'une mission de cinq jours à l'été 1978 sur l'estuaire moyen du St-Laurent. Cet estuaire d'une longueur d'environ 170 km permet de couvrir toute la gamme de salinité de 0 à 33‰. Au cours de cette mission, nous avons prélevé et analysé 188 couples d'échantillons. Le tableau 1 montre deux exemples types des analyses effectuées. pH_1 et pH_2 représentent les mesures de pH effectuées sur deux sous-échantillons provenant de la même bouteille de prélèvement. L'écart au pH moyen (δpH) demeure toujours très faible et ne semble pas lié à la salinité de l'échantillon. D'une part le deuxième groupe de mesures (*b*) ne montre pas d'écarts plus grands que le premier malgré des salinités beaucoup plus faibles et d'autre part un changement brusque de salinité entre deux prélèvements successifs (5.707‰ à 5 m et 13.746‰ à 10 m dans le groupe *b*) n'a pas occasionné un déséquilibre des électrodes et ne semble pas avoir affecté la fidélité de la méthode.

L'histogramme des écarts (δpH) pour les 188 couples est tracé à la figure 1. Le calcul montre que 95.5% des couples possèdent un écart (δpH) égal ou inférieur à ±0.005 unité de pH, tandis qu'un seul couple dépasse la marge de ±0.01 unité de pH. Ces résultats montrent que la fidélité de cette méthode, i.e., la capacité de fournir deux fois la même lecture pour deux sous-échantillons identiques, est comparable à celle rapportée par Pytkowicz et al. (1966).

La dérive des électrodes — En plus de la fidélité, il est nécessaire d'examiner la capacité de la méthode de fournir des résultats cohérents, comparables entre eux pour fin d'interprétation sur une période de temps relativement longue. L'utilisation du tampon TRIS et la mesure de son pH pendant les cinq jours de la mission nous fournissent deux indications précieuses à ce sujet. Pour cette interprétation, on doit considérer le tampon TRIS comme un échantillon d'eau estuarienne de salinité moyenne dont le pH est *invariable* dans le temps et supposer que les électrodes se comportent de la même façon avec ce tampon qu'avec n'importe quel échantillon prélevé en mer. La figure 2 montre l'ensemble des 41 mesures de pH effectuées sur la solution de tampon TRIS pendant les 120 h de mission en mer.

TABLEAU 1. Deux exemples types de séries de mesures effectuées dans l'estuaire moyen du St-Laurent.

Profondeur (m)	Salinité ‰	pH_1 (25°C)	pH_2 (25°C)	pH moyen (25°C)	Écart au pH moyen (δpH)
	a) position: 48°08.8' N, 69°32.9' W				
0	26.449	7.802	7.800	7.801	±0.001
5	26.786	7.788	7.787	7.788	0.001
9	27.183	7.756	7.766	7.761	0.005
23	29.640	7.728	7.730	7.729	0.001
46	31.390	7.711	7.713	7.712	0.001
69	31.846	7.721	7.720	7.721	<0.001
92	32.272	7.733	7.734	7.734	<0.001
	b) position: 47°12.2' N, 70°35.8' W				
0	04.017	8.037	8.035	8.036	0.001
5	05.707	7.920	7.920	7.920	0.000
10	13.746	7.776	7.777	7.777	<0.001
15	17.069	7.766	7.769	7.768	0.002

Ces résultats conduisent à deux observations majeures:

1) La dérive totale a été de −0.03 pH pour les cinq jours de mission, soit −0.006 pH par jour d'utilisation. Cette dérive est réelle et non négligeable à partir du deuxième jour et implique qu'une correction adéquate soit appliquée à toutes les valeurs de pH obtenues afin de rendre les données comparables entre elles sur toute la durée de la mission. Quoique ce type de dérive n'ait été mentionné antérieurement que par Zirino (1975), nous croyons qu'il ne s'agit pas d'un phénomène isolé et propre à notre série de mesures mais d'un problème inhérent à la nature même des électrodes classiques utilisées pour la mesure du pH. Le phénomène semble être attribuable à une modification lente et irréversible du potentiel de jonction causée par l'accumulation probable de substances parasites à la surface de la paroi de verre de l'électrode indicatrice (Bates 1975). Les

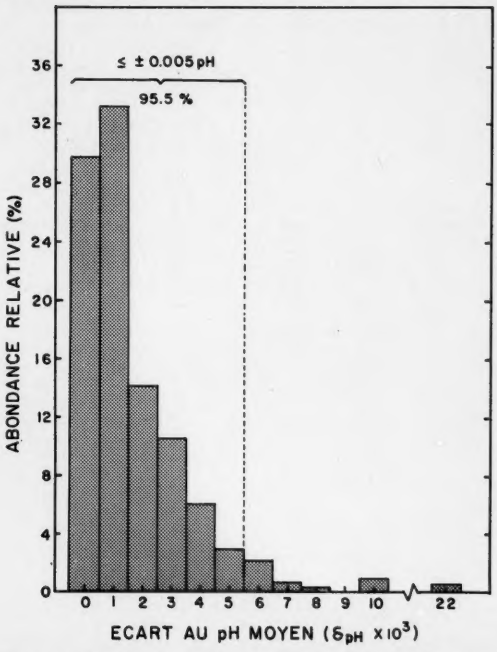

FIG. 1. Distribution de l'abondance relative de l'écart au pH moyen (δpH) calculée pour les 188 couples analysés dans les eaux estuariennes du St-Laurent.

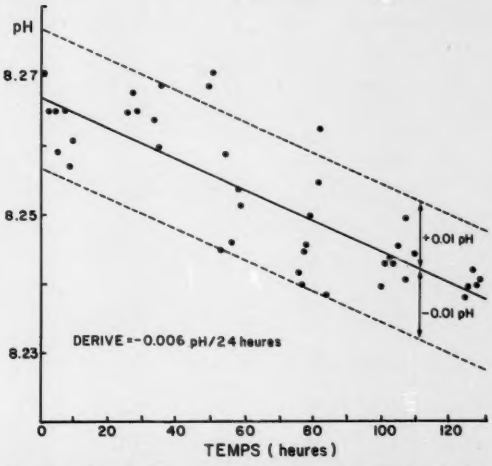

FIG. 2. Distribution des 41 mesures du pH de l'étalon secondaire (tampon TRIS à 18‰) en fonction du temps d'utilisation des électrodes en mission.

modifications étant faibles et imprévisibles sur une période de 24 h, il nous apparaît imprudent d'ajuster l'étalonnage du pHmètre pendant la mission; au retour, chaque valeur mesurée est recalée en fonction de la dérive enregistrée (fig. 2).

2) Le calcul statistique montre que 93% des mesures du tampon TRIS (fig. 2) se situe dans un corridor de ±0.010 unité de pH autour de la droite de régression calculée sur l'ensemble des mesures. Ce résultat peut être interprété comme une mesure de la précision de la méthode car il regroupe à la fois l'erreur analytique (la fidélité) présente dans chaque valeur et l'écart à la valeur moyenne réelle du pH du tampon TRIS en tenant compte de la dérive déjà observée. La précision de ±0.010 unité de pH observée ici pour une longue série de mesures en milieu estuarien est comparable à celle obtenue par Johnson et al. (1975). Ceux-ci ont montré par l'étude systématique de différents couples d'électrodes verre-calomel appliqué à l'analyse d'une eau de mer artificielle à 34.720‰ que la précision était de ±0.010 unité de pH avec un intervalle de confiance de 95%. Les résultats des mesures de pH en milieu estuarien doivent donc être interprétés avec certaines précautions et toute différence inférieure à 0.20 unité de pH entre deux échantillons distincts ne peut être considérée comme significative.

Nous sommes conscients que l'usage des tampons NBS nous amène à définir implicitement une échelle de pH qui inclut des potentiels de jonction qui peuvent varier avec la force ionique des eaux estuariennes. Les résultats obtenus par cette méthode ne permettent pas des interprétations thermodynamiques rigoureuses. Cependant, nous avons élaboré cette méthode dans le but de définir un protocole pratique et opérationnel nous permettant de mesurer avec précision les variations de pH en milieu estuarien et de relier les valeurs obtenues aux caractéristiques océanographiques du milieu.

Il n'existe à ce jour pratiquement aucune série de donnés importantes sur la variabilité du pH en milieu estuarien (Morris 1978). Pourtant, de telles données pourraient être d'une grande utilité à de nombreux chercheurs intéressés aux problèmes de distribution des espèces protolytiques (carbonates, borates, phosphates ...) et aux phénomènes de précipitation, de complexation organo-métallique, d'adsorption et de floculation encore très peu connus en milieu estuarien. L'intégration d'une méthode simple de la mesure du pH à la routine des analyses chimiques et biologiques des missions en milieux estuarien et côtier pourrait contribuer à combler cette lacune.

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ALMGREN, T., D. DYRSSEN ET M. STRANDBERG. 1975. Determination of pH on the moles per kg seawater scale (M_w). Deep-Sea Res. 22: 635–646.

BATES, R. 1975. pH scales for seawater, p. 313–338. *Dans* E. D. Goldberg [éd.] The nature of seawater, Dahlem Konferenzen, Pergamon Press, Berlin.

CARRITT, D. E. ET J. H. CARPENTER. 1966. Comparison and evaluation of currently employed modifications of the Winkler Method for determining oxygen in seawater. J. Mar. Res. 24: 286–318.

HANSSON, I. 1972. A new set of pH scales and standard buffers for seawater. Deep-Sea Res. 20: 479–491.

JOHNSON, K. S., R. VOLL, C. A. CURTIS ET R. M. PYTKOWICZ. 1977. A critical examination of the NBS pH scale and the determination of titration alkalinity. Deep-Sea Res. 24: 915–926.

MORRIS, A. W. 1978. Chemical processes in estuaries: The importance of pH and its variability, 15: 179–187. *Dans* W. E. Krumbein [éd.] Environmental bio-geo-chemistry and geomicrobiology, vol. 1: The aquatic environment. Ann Arbor Sci. Publishers Inc., Ann Arbor, Mich.

PARK, K. 1968. The processes contributing to the vertical distribution of the apparent pH in the northeastern Pacific Ocean. J. Oceanogr. Soc. Korea 3: 1–7.

PYTKOWICZ, R. M., D. R. KESTER ET B. C. BURGENER. 1966. Reproducibility of pH measurements in seawater. Limnol. Oceanogr. 11: 417–418.

ZIRINO, A. 1975. Measurement of the apparent pH of seawater with a combination microelectrode. Limnol. Oceanogr. 20: 654–657.

Stimulation of Nitrate Uptake and Photosynthesis by Molybdenum in Castle Lake, California

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AXLER, R. P., R. M. GERSBERG, AND C. R. GOLDMAN. 1980. Stimulation of nitrate uptake and photosynthesis by molybdenum in Castle Lake, California. Can. J. Fish. Aquat. Sci. 37: 707–712.

The uptake rates of $^{15}NO_3$ and $^{14}CO_2$ by the natural phytoplankton community at Castle Lake, California, were measured in situ as responses to 5 $\mu g \cdot L^{-1}$ additions of molybdenum. Stimulation of both nitrate uptake and photosynthesis occurred in water samples containing only relatively high amounts of nitrate. This response to added molybdenum disappeared as the growing season progressed and nitrate was depleted in the euphotic zone. Although molybdenum stimulated nitrate uptake by 55% in water collected from the lower euphotic zone, it did not increase the rate of CO_2 uptake because at that depth the rate of photosynthesis was most limited by light intensity and not by nitrogen. An analysis of molybdenum bioassays from 1959 to 1963 is integrated with these findings and points to the importance of molybdenum for phytoplankton growth during the early portion of the growing season when nitrate concentrations in the euphotic zone are maximal.

Key words: molybdenum, nitrate, nitrate uptake, micronutrient bioassays

AXLER, R. P., R. M. GERSBERG, AND C. R. GOLDMAN. 1980. Stimulation of nitrate uptake and photosynthesis by molybdenum in Castle Lake, California. Can. J. Fish. Aquat. Sci. 37: 707–712.

Les taux d'absorption de $^{15}NO_3$ et $^{14}CO_2$ par la communauté phytoplanctonique naturelle dans le lac Castle, en Californie, ont été mesurés in situ comme réactions à des additions de 5 $\mu g \cdot L^{-1}$ de molybdène. L'absorption de nitrates et la photosynthèse sont toutes deux stimulées dans des échantillons d'eau contenant seulement des quantités relativement fortes de nitrates. Cette réaction à une addition de molybdène disparaît à mesure que la saison avance et que les nitrates s'épuisent dans la zone euphotique. Bien que le molybdène stimule à 55 l'absorption de nitrates dans de l'eau recueillie à la partie inférieure de la zone euphotique, il n'accélère pas le taux d'absorption de CO_2, car à cette profondeur, le taux de photosynthèse est surtout limité par l'intensité lumineuse et non par l'azote. Nous intégrons dans ces résultats une analyse d'essais biologiques au molybdène de 1959 à 1963, d'où ressort l'importance du molybdène dans la croissance du phytoplancton pendant la première partie de la saison de croissance, quand les concentrations de nitrates dans la zone euphotique sont maximales.

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UTILIZING the ^{14}C-bioassay technique, Goldman (1960, 1964, 1967, 1978) showed significant increases in primary production with addition of molybdate and discovered that this trace element could limit productivity at Castle Lake, California. Hutchinson (1957) had earlier suspected the importance of molybdenum in aquatic systems due to its low concentration in freshwaters and its role in the nitrogen metabolism of microorganisms. In the decade following this discovery, numerous lakes were found to have very low concentrations of molybdenum (Bachmann and Goldman 1964; Bradford et al. 1968; Goldman 1964; Groth 1971) and also to exhibit increased rates of photosynthesis after addition of this metal to bioassay samples (Goldman 1964, 1972; Allen 1972).

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Field investigations regarding the functional role of molybdenum in lake ecosystems are sparse. Barsdate (1963) reported seasonal variations in the dissolved molybdenum concentration of eutrophic Sanctuary Lake, Pennsylvania, and observed a weak inverse correlation between cell density and molybdenum concentration. Dumont (1972) described the annual cycle of soluble Mo in a eutrophic pond in which the trace element appeared to be depleted during the major portion of the year by both biological uptake (and subsequent sedimentation) and by chemical precipitation with ferric iron. He pointed to the importance of sediment redox potential in regulating the concentration of dissolved Mo in the water column, as had been previously suggested by Goldman (1967) for Castle Lake.

When nitrogen is taken up in oxidized form by cells, as nitrate or nitrite, it must first be reduced to ammonium before being assimilated into organic compounds. The first evidence for the specific role of

molybdenum in the metabolism of microorganisms came from Steinberg (1937), who linked the metal to nitrate assimilation in the fungus *Aspergillus niger*. Studies by Arnon et al. (1955), Ichioka and Arnon (1955), and Wallen and Cartier (1975), using laboratory cultures of algae, demonstrated that the metal was essential for growth only when nitrate was the sole source of nitrogen in the growth medium. They further showed that chlorophyll synthesis and nitrate assimilation were depressed in molybdenum deficient cells. Additional investigations have shown that the nitrate reductase enzyme complex is synthesized (in the presence of nitrate) with or without molybdenum; however, the presence of molybdenum is required for enzyme activity (Notton and Hewitt 1971; Aparicio et al. 1971; Vega et al. 1971).

Studies concerning the specific role of molybdenum in natural ecosystems are scarce. In algae its role is limited to the processes of nitrate assimilation and nitrogen fixation. However, significant numbers of cyanobacteria containing heterocysts are never found in the water column at Castle Lake, and therefore the importance of this micronutrient in this lake must be related to its function in nitrate assimilation. In this paper the results are reported of experiments performed in Castle Lake, California, utilizing the uptake of $^{15}NO_3$ as a specific assay for delineating the role that molybdenum plays in regulating nitrate uptake. A reevaluation of previously reported ^{14}C-bioassay data is also presented and integrated with the ^{15}N results to evaluate the importance of molybdenum and nitrate to phytoplankton production in Castle Lake.

Methods — Water samples were collected using a nonmetallic sampler and filtered through 102-μm netting to remove zooplankton. Soluble nitrate was determined by a modification of the hydrazine reduction method described by Mullin and Riley (1955) and Kamphake et al. (1967).

Carbon-14 enrichment bioassays for the years 1959–63 were performed as described in Goldman (1963). Enrichment bioassays performed in 1978 differed in that water samples were preincubated with 5 μg Mo·L^{-1} (as Na_2MoO_4) for 24 h prior to ^{14}C inoculation. Both controls and Mo-enriched samples were resuspended at the sampling depth in large bottles to ensure sufficient time for either in vivo activation of existing nitrate reductase or de novo synthesis of the enzyme (see Notton and Hewitt 1971; Vega et al. 1971). Four or five replicate samples were then transferred to 125-mL Pyrex bottles, inoculated with 92–370 KBq of $H^{14}CO_3^-$, and resuspended for 4 h near midday. The seston was then immediately concentrated onto Millipore 0.45-μm membrane filters under low vacuum and counted with a thin window gas flow Geiger–Müller counter to 10 000 counts.

The ^{15}N tracer method, first introduced for use in lake water experiments by Neess et al. (1962) and Dugdale and Dugdale (1965), was used to measure nitrate uptake. Water enclosed in 2-L glass bottles was inoculated with 99 atom-% $^{15}NO_3^-$ and incubated in situ for 24 h. As for the ^{14}C experiments, all samples were given a 16- to 24-h pretreatment with Mo. Since previous measurements had indicated that the rate of nitrate uptake was extremely low

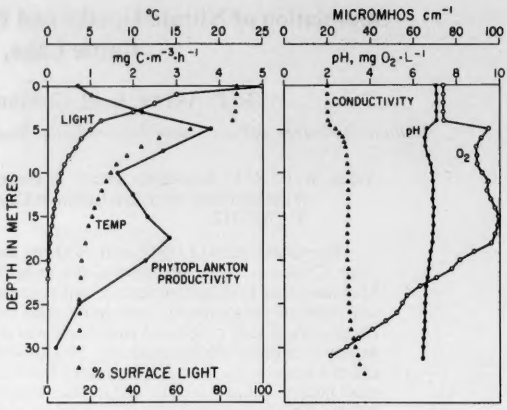

FIG. 1. General midsummer limnological characteristics of Castle Lake.

(Gersberg et al. 1978) the amount of $^{15}NO_3^-$ was adjusted to yield a final NO_3^--N concentration of about 50 μg N·L^{-1} to ensure a measurable rate of uptake. Therefore, the resulting rates approximate maximum uptake rates for nitrate. Experiments were terminated by low vacuum filtration through precombusted GF/C filters which were dried and frozen until analysis. Samples were prepared for mass spectrometry (UTI-model 100) according to the procedure of Günther et al. (1966). Particulate nitrogen was determined directly from ^{15}N filters by cross-calibrating gas pressures in the mass spectrometry system to particulate nitrogen in parallel samples analyzed using a Carlo Erba 1104 elemental analyzer.

Results and discussion — Castle Lake is a small, meso-oligotrophic lake located at an elevation of 1706 m in the Klamath Mountains of northern California. The general limnological characteristics for a typical midsummer day are presented in Fig. 1. The euphotic zone (0–25 m) is characterized by a gradual depletion of nitrate after the spring thaw and levels remain low (generally <5 μg N·L^{-1}) until the fall overturn (Fig. 2). The years 1977 and 1978 represent low and high "nitrate" years, respectively, in that nitrate had virtually disappeared from the euphotic zone by mid-June 1977 whereas in 1978 levels of nitrate were not severely depleted until well into August. The persistence of significant quantities of nitrate well into the growing season of 1978 was a consequence of a late ice-thaw (June 8), heavy snowpack, and high spring runoff together with a large release of ammonium (and subsequent nitrification to produce nitrate) from the sediment during the summer and fall of the previous year. Gersberg (1977) showed that the concentration of nitrate in the lower hypolimnion is determined by the difference between nitrate production (nitrification of ammonium released from both the sediment and from mineralization of sedimenting seston) and nitrate removal (denitrification) processes which are in turn

TABLE 1. Summary of molybdenum enriched ^{14}C-bioassays using epilimnetic water. Comparison of all "early" season experiments to all "late" season experiments by *t*-tests.

	% above control ($\bar{x}$ = SD)		
	Pooled experiment	Pooled seasons	*P*
June 25	21.3±10.6		
June 29	88.9±12.6	x_1 = 72.1±55.9 (Early)	
July 02	131.4±55.1		
1959			<0.01
July 17	−9.6±10.3		
August 08	−6.4±4.9	x_2 = −8.0±7.4 (Late)	
June 18	8.9±9.8	x_3 = 8.9±9.8 (Early)	
1960			<0.01
July 20	−4.0±5.2		
August 26	−6.9±1.8	x_4 = −5.2±4.1 (Late)	
June 20	5.3±8.7		
June 22	6.4±8.1	x_5 = 5.9±9.5 (Early)	
July 07	6.1±14.7		
1962			<0.02
July 14	−8.3±7.6		
July 17	−6.7±8.6	x_6 = −7.5±7.3 (Late)	
July 08	31.6±6.7	x_7 = 31.6±6.7 (Early)	
1963			<0.01
July >23	Molybdenum fertilization of Castle Lake (no positive effect again until 1966)		

regulated by the concentration of oxygen in these bottom waters. The early depletion of nitrate from the euphotic zone in 1977 occurred because the winter of 1976–77 was unusually mild, producing less snow, less spring runoff, and an earlier ice-thaw (before May 1) (and therefore, an earlier onset of nitrate depletion due to phytoplankton assimilation) than most years. In addition, spring mixing was incomplete in 1977, thereby resulting in a lower initial pool of nitrate in the euphotic zone. This pattern of gradual nitrate depletion as the summer progresses appears to be typical of many lakes (Liao and Lean 1978a, b; Burns 1976) and suggests that nitrate is most important as a source of nitrogen for phytoplankton growth in the first portion of the growing season.

After the discovery that molybdenum was a nutrient

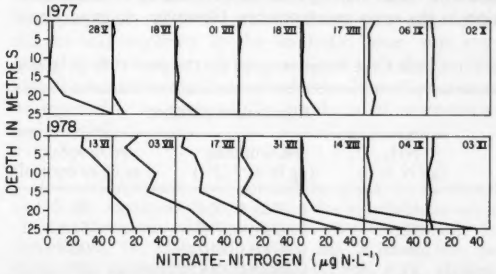

FIG. 2. Vertical profiles of NO_3^--N ($\mu g\ N \cdot L^{-1}$) in Castle Lake for the years 1977 and 1978.

limiting the growth of phytoplankton at Castle Lake, experimental additions of molybdate were made to the lake in 1963 and 1969 in an attempt to increase biological productivity. Large increases in benthic, zooplankton, and fish production were noted but any stimulation of primary production was masked by the large daily and seasonal fluctuations characteristic of this lake, although changes in phytoplankton species composition and succession rate were observed (Goldman 1972; Jassby and Goldman 1974). Current research regarding the dynamics of inorganic nitrogen uptake by Castle Lake phytoplankton has indicated that subsequent to thermal stratification and nitrate depletion, the algal community derives most of its nitrogen in the form of ammonium, which is rapidly recycled via mineralization and zooplankton excretion (Gersberg et al. 1980; Axler 1979). The importance of nitrate as a source of nitrogen for algal growth appears to be restricted to the early portion of the growing season and to the fall when lake-mixing processes and increased precipitation and stream inflow increase available nitrate in the photic zone.

In Table 1 the results of molybdenum enriched ^{14}C-bioassays using epilimnetic water (3 m) from Castle Lake during the ice-free periods of 1959–1963 are summarized. The data for each experiment were pooled, and so each number represents the mean effect exerted by enrichments of 1–10 $\mu g\ Mo \cdot L^{-1}$ after 1–5 d incubation. There are striking differences between the early (pre-July 10) and late (post-July 10) portions of the growing period in that molybdenum is stimulating only in the early summer. The difference between pooled means of early versus late-summer molybdenum enrichment experiments was statistically significant (*t*-test $P < 0.01$). There have been no significant changes in watershed vegetation or human-related factors in the past 20 yr which could change the nutrient loading characteristics of the lake and, therefore, it is reasonable to assume that in these years nitrate was depleted according to the pattern found in recent years (it should be stated again that 1977 and 1978 represent very low and very high "nitrate" years, respectively, and that the pool of epilimnetic nitrate usually persists until mid-July). This bioassay analysis points to the importance of molybdenum early in the growing season when significant nitrate is available for phytoplankton assimilation. The molybdenum content of the water has now declined to prefertilization levels (<0.2 $\mu g\ Mo \cdot L^{-1}$ in 1975, as measured using the X-ray fluorescence method of Elder et al. 1975) and therefore, Castle Lake is once again an ideal natural environment in which to investigate the effect of molybdenum on primary productivity.

Our hypothesis is that molybdenum can limit phytoplankton productivity through its role in the assimilation of nitrate, and therefore, its importance is expected to be restricted to only those times of the year (or depths) when nitrate concentrations are not so low as to limit severely the rate of nitrate assimilation. The

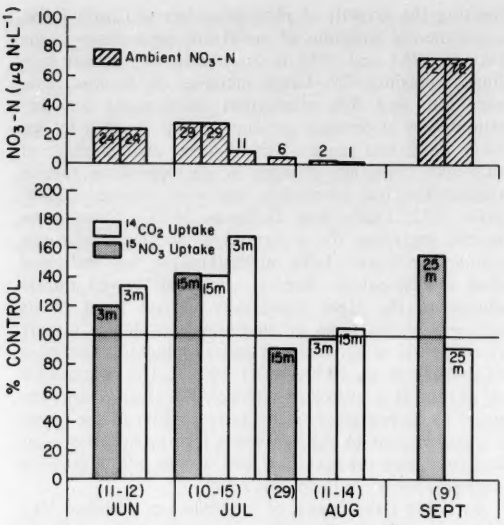

FIG. 3. Results of $^{15}NO_3^-$ and $^{14}CO_2$ molybdenum enrichment bioassays expressed as % of control, and ambient nitrate concentration. The numbers in the lower bars indicate sampling depths.

results of the $^{15}NO_3^-$ and $^{14}CO_2$ enrichment bioassays for the major part of the 1978 growing season, are summarized graphically in Fig. 3.

Nitrate uptake increased upon addition of molybdenum at 3 m in June ($P < 0.08$), at 15 m in early July ($P < 0.30$), and at 25 m in September ($P < 0.02$). Photosynthesis was significantly stimulated ($P < 0.01$) in water samples from 3 m in June and from 3 and 15 m during June and early July. It is apparent that phytoplankton populations responded to addition of molybdenum only when significant amounts of nitrate were available in the water prior to the addition of ^{15}N. The concentration of nitrate in the mid-epilimnion (3 m) had decreased from 11 $\mu g\,N \cdot L^{-1}$ to about 5 $\mu g\,N \cdot L^{-1}$ during the 3rd wk in July and from 12 $\mu g\,N \cdot L^{-1}$ to 4 $\mu g\,N \cdot L^{-1}$ in the mid-hypolimnion (15 m) during the last week in July. The stimulation of either $^{15}NO_3^-$ or $^{14}CO_2$ uptake in response to molybdenum enrichment disappears after these time periods. The lack of ^{14}C stimulation after nitrate levels were depleted is consistent with the bioassay results presented in Table 1. However, the absence of nitrate uptake stimulation at 15 m on July 29 is curious since the water is enriched with 50 $\mu g\,N \cdot L^{-1}$ (as $^{15}NO_3^-$), and this suggests several possibilities.

The first is that nitrate concentration had decreased below induction level for the enzyme nitrate reductase (NR). Eppley et al. (1969) reported such an effect in marine water samples when nitrate levels were of the order of 7 $\mu g\,N \cdot L^{-1}$ or less. In our experiment this would mean that at least some species of algae were still able to take up nitrate (because nitrate uptake was measurable in both controls and Mo-treated samples), but that a molybdenum deficiency was no longer present.

A second explanation is that another factor was limiting NR activity to a greater degree than molybdenum. Numerous investigators have reported that ammonium can repress NR activity and suppress nitrate uptake in both laboratory cultures and natural populations of algae (Morris and Syrett 1963; Syrett and Morris 1963; Eppley et al. 1969; Herrera et al. 1972; Bates 1976; Conway 1977). Table 2 presents the results of experiments in which water samples exhibited a reduction of 10–64% in their uptake of nitrate upon enrichment with ammonium. Levels of ammonium in the euphotic zone of Castle Lake are consistently low (0–5 $\mu g\,N \cdot L^{-1}$) during the entire summer; however, rates of ammonium turnover and uptake have been shown to be relatively high because of rapid regeneration via zooplankton excretion and mineralization (Axler 1979). Because it is the intracellular ammonium concentration (or amino acid pool) which ultimately affects the NR activity (Conway 1977, cf. Healey 1973), it is possible that this type of mechanism is, in fact, occurring during mid-late summer when rates of ammonium uptake are maximal relative to nitrate uptake, despite low concentrations of ambient ammonium. The net result would be to mask effects due to molybdenum.

Unfortunately we do not have ^{15}N data for epilimnetic water during this period because of a malfunction in the mass spectrometer. However, an experiment

TABLE 2. Effect of added ammonium on the rate of nitrate uptake in Castle Lake water samples for the year 1978 ($\bar{x} \pm$ SD).

Date (depth)	Control		Ammonium enriched		
	Ambient NH_4 ($\mu g\,N \cdot L^{-1}$)	NO_3-uptake ($\mu g\,N \cdot L^{-1} \cdot d^{-1}$)	NH_4 ($\mu g\,N \cdot L^{-1}$)	NO_3-uptake ($\mu g\,N \cdot L^{-1} \cdot d^{-1}$)	NO_3-uptake (as % of control)
June 15 (3 m)	1.0	0.085 ± 0.013	12.9	0.076 ± 0.004	89
June 29 (3 m)	2.9	0.065 ± 0.008	50.9	0.035 ± 0.016	54
July 01 (3 m)	3.0	0.087	12.5–26.8	0.078 ± 0.010	90
July 29 (15 m)	3.8	0.227 ± 0.056	49.1	0.082 ± 0.018	36
August 14 (20 m)	1.9	0.239 ± 0.020	47.3	0.169 ± 0.001	71

conducted on 3 m water in September 1976, when the ambient nitrate concentration was also depleted (3 μg $N \cdot L^{-1}$), showed no increase in nitrate uptake due to molybdenum addition (R. P. Axler 1976, unpublished data). This experiment utilized the carrier-free radiotracer $^{13}NO_3^-$ (as described by Gersberg et al. 1978) which enabled us to conduct the experiment at both natural and enriched (50 $\mu g\, N \cdot L^{-1}$) substrate concentrations and therefore, again pointed to some factor other than molybdenum which was limiting the rate of nitrate uptake.

The results of the September experiments are interesting in that NO_3^- uptake was stimulated 55% ($P < 0.02$) whereas CO_2 uptake was inhibited slightly (9%, $P > 0.3$). Water for this experiment was collected from 25 m, near the lower limit of the photic zone (light intensity ~0.3% of surface). Although ambient nitrate concentration was high (72 $\mu g\, N \cdot L^{-1}$), in situ primary productivity was only about 1 $\mu g\, C \cdot L^{-1} \cdot d^{-1}$ and was light limited. It seems likely that for these cells, sufficient nitrogen is already available to meet their physiological requirements for photosynthesis despite low Mo, and consequently the ^{14}C-experiments do not show a positive response. In situ uptake of ammonium at 25 m, as measured using $^{15}NH_4^+$ on September 4, was approximately twice that of nitrate (1.0 $\mu g\, NH_4^+\text{-}N \cdot L^{-1} \cdot d^{-1}$ versus 0.5 $\mu g\, NO_3^-\text{-}N \cdot L^{-1} \cdot d^{-1}$). Therefore, even a 55% stimulation of nitrate uptake would only increase the total daily uptake of inorganic nitrogen by 19%. The mean photosynthetic rate at 25 m during the period of September 1–19, 1978 was $4.7 \pm 0.4\, \mu g\, C \cdot L^{-1} \cdot d^{-1}$. One can then calculate a carbon to nitrogen ($NH_4^+\text{-}N + NO_3^-\text{-}N$) uptake ratio (by weight) of 3.1 which does not suggest that the community at this depth is deficient in nitrogen, and one would not expect Mo to stimulate the rate of photosynthesis.

The use of $^{15}NO_3^-$ as a tracer for determining the influence of molybdenum on nitrate uptake kinetics in aquatic systems was first suggested by Dugdale (1967). Using this approach we have demonstrated the key role that can be played by this micronutrient in the nitrogen metabolism of natural populations of Castle Lake phytoplankton, and our data supports the results of previous ^{14}C-enrichment bioassays (Table 1). The disappearance of the molybdenum response, after nitrate was depleted in the euphotic zone, was suggested to have occurred as a result of a decline in NR activity due either to "below-induction" levels of nitrate or repression by intracellular products of ammonium assimilation.

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Allen, H. L. 1972. Phytoplankton photosynthesis, micronutrient interactions, and inorganic carbon availability in a soft-water Vermont lake, p. 63–84. *In* G. E. Likens [ed.] Nutrients and eutrophication. Am. Soc. Limnol. Oceanogr. Spec. Symp. 1.

Aparicio, P. J., J. Cardenas, W. G. Zumft, J. M. Vega, J. Herrera, A. Paneque, and M. Losada. 1971. Molybdenum and iron as constituents of the enzymes of the nitrate reducing system from *Chlorella*. Phytochemistry 10: 1487–1496.

Arnon, D. I., P. S. Ichioka, G. Wessel, A. Fujiwara, and J. T. Wooley. 1955. Molybdenum in relation to nitrogen metabolism I. Assimilation of nitrate nitrogen by *Scenedesmus*. Physiol. Plant. 8: 538–551.

Axler, R. P. 1979. Inorganic nitrogen uptake by phytoplankton in Castle Lake, California. Ph.D. thesis, Univ. California, Davis, CA. 150 p.

Bachmann, R., and C. R. Goldman. 1964. The determination of microgram quantities of molybdenum in natural waters. Limnol. Oceanogr. 9: 143–146.

Barsdate, R. J. 1963. The biogeochemistry of molybdenum in lakes and in the sea. Ph.D. dissertation, Univ. of Pittsburgh, Pittsburgh, PA. 92 p.

Bates, S. S. 1976. Effects of light and ammonium on nitrate uptake by two species of estuarine phytoplankton. Limnol. Oceanogr. 21: 212–218.

Bradford, G. R., F. L. Blair, and V. Hunsaker. 1968. Trace and major element content of 170 High Sierra lakes. Limnol. Oceanogr. 13: 526–530.

Burns, N. M. 1976. Temperature, oxygen, and nutrient distribution patterns in Lake Erie, 1970. J. Fish. Res. Board Can. 33: 485–511.

Conway, H. L. 1977. Interactions of inorganic nitrogen in the uptake and assimilation by marine phytoplankton. Mar. Biol. 39: 221–232.

Dugdale, R. C. 1967. Nutrient limitation in the sea: dynamics, identification, and significance. Limnol. Oceanogr. 12: 685–695.

Dugdale, V. A., and R. C. Dugdale. 1965. Nitrogen metabolism in lakes III. Tracer studies of the assimilation of inorganic nitrogen sources. Limnol. Oceanogr. 10: 53–57.

Dumont, H. J. 1972. The biological cycle of molybdenum in relation to primary production and water bloom formation in a eutrophic pond. Verh. Int. Ver. Limnol. 18: 84–92.

Elder, J. F., S. K. Perry, and F. P. Brady. 1975. Application of energy-dispersive X-ray fluorescence to trace metal analysis of natural waters. Environ. Sci. Technol. 9: 1039–1042.

Eppley, R. W., J. L. Coatsworth, and L. Solorzano. 1969. Studies of nitrate reductase in marine phytoplankton. Limnol. Oceanogr. 14: 194–205.

Gersberg, R. M. 1977. Denitrification studies in Castle Lake, California. Ph.D. thesis, Univ. California, Davis, CA. 103 p.

Gersberg, R. M., R. P. Axler, and C. R. Goldman. 1980. Isotope studies of nitrogen transformations in Castle Lake, California. Proc. FAO/IAEA meeting, August 28, 1978, Vienna, Austria. (In press)

Gersberg, R. M., R. P. Axler, K. Krohn, N. Peek, and C. R. Goldman. 1978. Nitrate uptake by phytoplankton: measurements utilizing the radioisotope ^{13}N. Verh. Int. Ver. Limnol. 20: 388–392.

GOLDMAN, C. R. 1960. Molybdenum as a factor limiting primary productivity in Castle Lake, California. Science 132: 1016–1017.

1963. The measurement of primary productivity and limiting factors in freshwater with carbon-14, p. 103–113. *In* M. S. Doty [ed.] Proc. Conf. on Primary Productivity Measurement, Marine and Freshwater, U.S.A.E.C., Div. Tech. Inform. Rep. TID-7633.

1964. Primary productivity and micronutrient limiting factors in some North American and New Zealand lakes. Verh. Int. Ver. Limnol. 15: 365–374.

1967. Molybdenum as an essential micronutrient and useful watermass marker in Castle Lake, California, p. 229–238. *In* H. L. Golterman and R. S. Clymo [ed.] Chemical environment in the aquatic habitat. Proc. IBP Symp., Amsterdam and Nieuwersluis, October 1966. N.V. Noord-Hollandsche Uitgevers Maatschappij, Amsterdam, Holland.

1972. The role of minor nutrients in limiting the productivity of aquatic ecosystems, p. 21–38. *In* G. E. Likens [ed.] Nutrients and eutrophication. Am. Soc. Limnol. Oceanogr. Spec. Symp. 1.

1978. The use of natural phytoplankton populations in bioassay. Mitt. Int. Ver. Limnol. 21: 364–371.

GROTH, P. 1971. Untersuchungen über einige Spurenelemente in Seen. Arch. Hydrobiol. 68: 305–375.

GÜNTHER, H., H. G. FLOSS, AND V. H. SIMON. 1966. Ein Vereinfachtes verfahren zur ^{15}N-Bestimmung. Z. Anal. Chem. 218: 401–408.

HEALEY, F. P. 1973. Inorganic nutrient uptake and deficiency in algae. Crit. Rev. Microbiol. 3: 69–113.

HERRERA, J., A. PANEQUE, J. M. MALONADO, J. L. BAREA, AND M. LOSADA. 1972. Regulation by ammonia of nitrate reductase synthesis and activity in *Chlamydomonas reinhardi*. Biochem. Biophys. Res. Commun. 48: 996–1001.

HUTCHINSON, G. E. 1957. A treatise on limnology. Vol. 1. *In* Geography, physics and chemistry. Wiley, New York, NY. 1015 p.

ICHIOKA, P. S., AND D. I. ARNON. 1955. Molybdenum in relation to nitrogen metabolism. II. Assimilation of ammonia and urea without molybdenum by *Scenedesmus*. Physiol. Plant. 8: 552–560.

JASSBY, A. D., AND C. R. GOLDMAN. 1974. A quantitative measure of succession rate and its application to the phytoplankton of lakes. Am. Nat. 108: 688–693.

KAMPHAKE, L. J., S. A. HANNAH, AND J. M. COHEN. 1967. Automated analysis for nitrate by hydrazine reduction. Water Res. 1: 205–216.

LIAO, C. F.-H., AND D. R. S. LEAN. 1978a. Seasonal changes in nitrogen compartments of lakes under different loading conditions. J. Fish. Res. Board Can. 35: 1095–1101.

1978b. Nitrogen transformations within the trophogenic zone of lakes. J. Fish. Res. Board Can. 35: 1102–1108.

MORRIS, I., AND P. J. SYRETT. 1963. The development of nitrate reductase in *Chlorella* and its repression by ammonium. Arch. Mikrobiol. 47: 32–41.

MULLIN, J. B., AND J. P. RILEY. 1955. The spectrophotometric determination of nitrate in natural waters, with particular reference to sea water. Anal. Chim. Acta 12: 464–480.

NEESS, J. C., R. C. DUGDALE, V. A. DUGDALE, AND J. J. GOERING. 1962. Nitrogen metabolism in lakes. I. Measurement of nitrogen fixation with ^{15}N. Limnol. Oceanogr. 7: 163–169.

NOTTON, B. A., AND E. J. HEWITT. 1971. Incorporation of radioactive molybdenum into protein during nitrate reductase formation and effect of molybdenum on nitrate reductase and diaphorase activities of spinach (*Spinacea oleracea* L.). Plant Cell Physiol. 12: 465–477.

STEINBERG, R. A. 1937. Role of molybdenum in the utilization of ammonium and nitrate nitrogen by *Aspergillus niger*. J. Agric. Res. (Washington, D.C.) 55: 891–902.

SYRETT, P. J., AND I. MORRIS. 1963. The inhibition of nitrate assimilation by ammonium in *Chlorella*. Biochim. Biophys. Acta 67: 566–575.

VEGA, J. M., J. HERRERA, P. J. APARICIO, A. PANEQUE, AND M. LOSADA. 1971. Role of molybdenum in nitrate reduction by *Chlorella*. Plant Physiol. 48: 294–299.

WALLEN, D. G., AND L. D. CARTIER. 1975. Molybdenum dependence, nitrate uptake and photosynthesis of freshwater plankton algae. J. Phycol. 11: 345–349.

Variability of Dentin Deposits in *Tursiops truncatus*

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HUI, C. A. 1980. Variability of dentin deposits in *Tursiops truncatus*. Can. J. Fish. Aquat. Sci. 37: 712–716.

Dentin is deposited in approximately annual cycles in *Tursiops truncatus* for at least the first 11 yr. There were no consistent differences in the dentinal layer count between the left and right sides nor between the mandible and maxilla in the teeth of nine animals studied. The posterior teeth, however, have a greater number of growth layer groups (GLGs). The differences in the number of GLGs among teeth of the same individual increase unpredictably when there are more than about 15 GLGs in the posterior teeth. Only minimal age may be determined using dentinal counts.

Key words: age determination, dentin, dolphins, odontocetes, teeth, *Tursiops*

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HUI, C. A. 1980. Variability of dentin deposits in *Tursiops truncatus*. Can. J. Fish. Aquat. Sci. 37: 712–716.

La dentine se dépose en cycles d'approximativement un an, pendant au moins les 11 premières années de vie, chez *Tursiops truncatus*. Il n'y a pas de différences uniformes du nombre de couches de dentine entre les côtés gauche et droit, non plus qu'entre la mandibule et le maxillaire sur les dents de neuf sujets examinés. Les dents postérieures ont toutefois le plus grand nombre de groupes de couches de croissance (GLG, « growth layer groups »). Les différences de nombre de GLG parmi les dents d'un même individu augmentent de manière imprévisible quand il y a plus qu'environ 15 GLG sur les dents postérieures. Le dénombrement des couches de dentine donne seulement un âge minimal.

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THE bottlenose dolphin, *Tursiops truncatus*, is one of the most exploited species of small cetaceans in North American waters. It is used extensively for display and research. Knowing the ages of the individuals collected will permit estimates on the impact caused by removal of those individuals from the population and estimates on their fitness for their intended use.

Several techniques for determining age in mammals have been reported, and one of the most useful of them is examination of the cyclic dentinal deposits. The method has been applied to a variety of marine mammals including elephant seals (*Mirounga leonina*) (Laws 1953), harbor seals (*Phoca vitulina*) (Mansfield and Fisher 1960), grey seals (*Halichoerus grypus*) (Hewer 1964), sperm whales (*Physeter catodon*) (Ohsumi et al. 1962), bottlenose whales (*Hyperoodon ampullatus*) (Christensen 1973), white whales (*Delphinapterus leucas*) (Sergeant 1973), pilot whales (*Globicephala malaena*) (Sergeant 1962), common dolphins (*Delphinus delphis*) (Hui 1979), striped dolphins (*Stenella caeruleoalba*) (Kasuya 1972), spotted dolphins (*Stenella attenuata*) (Perrin et al. 1976), dusky dolphins (*Lagenorhynchus obscurus*) (Best 1976), and bottlenose dolphins (*Tursiops truncatus*) (Sergeant 1959; Sergeant et al. 1973).

The number of dentinal layers in the teeth of a *T. truncatus* specimen may be an accurate indicator of its chronological age. In captive-born animals of ages 2.5 and 0.5 yr, one dentin cycle was completed for each year (Sergeant 1959). In a subsequent study which used a larger sample including wild and captive specimens, Sergeant et al. (1973) concluded that one dentinal cycle was deposited each year.

Radiographs which I have taken of *Delphinus delphis* and *T. truncatus* show that at birth all the teeth are formed, although unerupted. If the general sequence of tooth eruption noted from my numerous observations of young specimens of *D. delphis* also applies to *T. truncatus*, the eruption sequence is related to the size of teeth at birth: the larger teeth erupt earlier. The posterior teeth, except the last two or three which are smaller, erupt before the anterior teeth and those of the maxilla before those of the mandible.

Development of the teeth of an individual occurs at different rates corresponding to the relative tooth size at the animal's birth. This differential growth may be the result of (1) teeth of different sizes reflecting growth increments of different sizes but of equal numbers, or (2) the growth increments may be the same size but growth ceases at different times in the different teeth. A combination of these two processes might also be taking place.

If dentinal layers are to be used as indicators of age in *T. truncatus*, their relationship to chronological age needs to be known. If the number of dentinal layers varies between teeth, the most reliable tooth (or teeth) for age indication needs to be determined. The present study was designed primarily to obtain an estimate of the variability of the number of layers among teeth of an individual. Because of the availability of material, it was also possible to reexamine in a small way the relationship of dentinal cycles to chronological age.

Materials and methods — In this report I adhere to the term "growth layer groups" as defined by Lockyer et al. (1980): "Growth layer groups (GLGs) are groups of incremental growth layers which may be recognized by virtue of a cyclic repetition, generally at constant or regularly changing relative spacing. . . ." Each of the growth layer groups I observed consisted of numerous "lines," "bands," "zones," or "laminae" distinguished as darker or lighter than its neighbor. These component structures varied in a number from one GLG to the next but were never more than 15 light–dark pairs. Each GLG was defined as ending in an exceptionally broader darker "line." Figure 1 illustrates what I considered to be the annual growth layer groups (see Perrin and Myrick (1980) for a more detailed description of *T. truncatus* teeth).

The GLG count was not considered determined until I could repeat it several times to my own satisfaction. Any biases I may have in interpretation should be consistent throughout the samples.

The *T. truncatus* specimens used in this study were all collected along the southeastern coast of the United States or born to animals collected there. They all were maintained in captivity but for various lengths of time. Presumably, the sex and diet of the individual have no influence on the relationship of anterior and posterior teeth to their dentin deposition.

Teeth from three specimens of *T. truncatus* were used to estimate the time represented by one GLG. Two teeth were collected 2.5 yr apart from one animal using the technique developed by Ridgway et al. (1975). One tooth

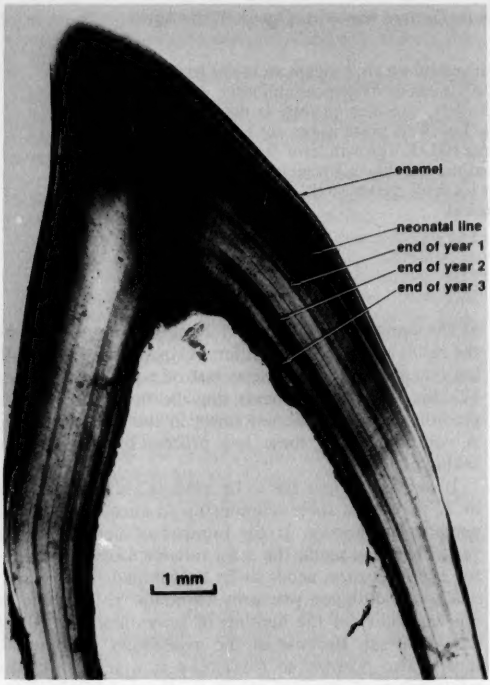

FIG. 1. Tooth from male *T. truncatus* which was born in September 1970 and died in February 1974. The dentinal layers believed to represent annual depositions are noted. The neonatal line is believed to be formed at birth.

was collected postmortem from each of two animals born in captivity: one which lived 3.4 yr and one which lived 3.3 yr.

In nine other animals, the number of GLGs in each tooth extracted from each individual was compared to the tooth containing the maximum number of GLGs for that individual. There were 21–24 teeth in each of the four jaw sections of the animals studied; the upper jaw sections tended to have two more teeth than the lower. In six of the animals, the 4th, 9th, 14th, and 19th teeth from each quarter of the mouth were extracted. Teeth from only the right mandible were extracted in two animals. Two teeth (4th and 19th) were extracted from each of four quarters in one animal. Teeth were counted from the anterior end of the jaw to the posterior.

After extraction, each tooth was scraped clean of all soft tissue and mounted on a small wooden block with 5-min epoxy (Devcon Corp., Danvers., Mass. 01923). Longitudinal sections, 0.38-mm thick, were cut from the center section using a low-speed saw (Beuhler Ltd., Evanston, IL 60204). After they were cut, the sections were washed with a weak solution of household ammonia to remove oil and residues, thoroughly rinsed with deionized water, and etched in 1–2% formic acid for 1–5 h. Etching time for teeth varied among individual animals but not among the teeth of a single animal. State of readiness was determined by inspection with the unaided eye. When distinct light and dark layers appeared with back lighting, the samples were rinsed with water and stored in buffered 10% formalin. These visible layers are the GLGs. The component structures which compose the GLGs were rarely distinguished by the unaided eye.

For examination, the sections were placed in water on a microscope slide and covered with a cover slip. A compound microscope employing transmitted light with a nearly closed diaphragm was used, with magnification at 37×. Manipulating the focus and condenser revealed more details in areas of particular interest than is obtainable from photographs.

The method used in this study is simple and can easily be conducted at the site of animal collection. Decalcification, staining, polarized light, and other methods, however, provide better detail enhancement (see Perrin and Myrick 1980 for comprehensive presentation) although they may also require more laboratory facilities.

Results — The teeth from the animals 3.3 and 3.4 yr old displayed three complete GLGs and an incomplete GLG (Fig. 1). More than two but less than three complete GLGs were deposited by the animal which had extractions 2.5 yr apart. When several teeth from each animal were compared, there were no consistent differences in the GLG count between the left and right sides nor between the mandibular and maxillary tooth sections. It appears, however, that anterior teeth cease depositing dentin after about 10–12 GLGs have been deposited (Fig. 2A) while the more posterior teeth may cease growth any time after about 15 GLGs (Fig. 2B). The number of dentinal GLGs in the anterior teeth have a correlation of 0.66 to the maximum number of dentinal GLGs obtained while the number of GLGs in the posterior teeth have a correlation of 0.87 (Kendall's Tau, Conover 1971).

Discussion — The specimens from *T. truncatus* of known ages demonstrate a distinct dentinal pattern. This pattern repeats itself in cycles whose number corresponds to the chronological ages of the animals. It is these cycles which define a growth layer group for purposes of age determination. The dentin in *T. truncatus* is discernible in ~1-yr increments at least through the first 11 yr. Previous studies of the regularity of dentinal deposition for *T. truncatus* report similar findings (Sergeant 1959; Sergeant et al. 1973). The deposits which compose the annual increment may or may not be formed at a regular rate. This study did not examine that aspect.

Within an older individual, different teeth have different numbers of dentinal GLGs. From birth to the deposition of 10–12 GLGs all the teeth appear to deposit dentinal layers with equal regularity. However, after this time the more anterior teeth cease depositing dentin while the posterior teeth continue. Anytime after depositing about 15 GLGs, the posterior teeth may cease deposition. The exact time a tooth ceases dentin deposition is not predictable (I have counted 32 GLGs in a posterior tooth from an ani-

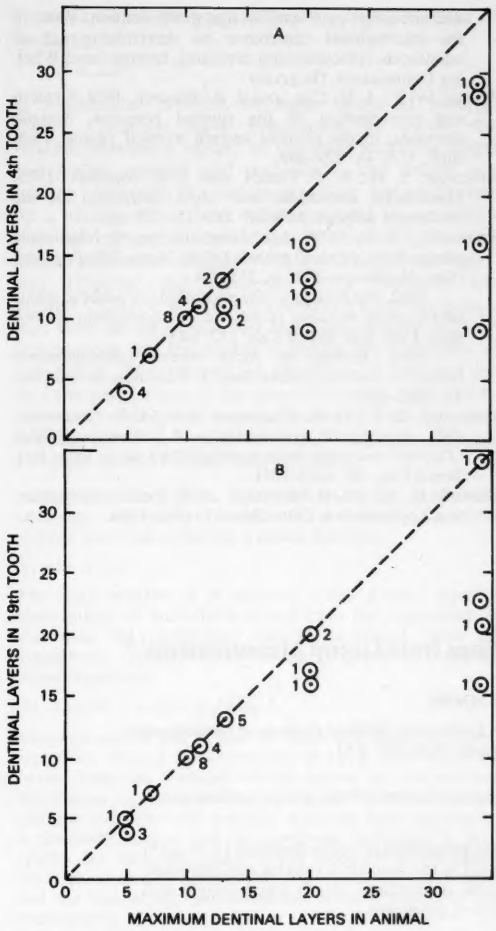

FIG. 2. Dentinal growth-layer-group counts from the anterior (part A) and posterior (part B) regions of the jaw relative to the maximum counts obtained from each animal. The numerals beside each point indicate the number of data which are represented by that point. If all the teeth of one animal had the same number of dentinal GLGs, all the points would occur on the broken line. Nine animals were examined.

mal not included in this study). As an animal grows older, the differences in the number of GLGs among the teeth increase, so that a single tooth from an older animal is a less reliable indicator of age than one from a younger animal. After deposition of about 15 GLGs in the posterior teeth (15th–19th) the indicated age must be viewed only as the minimum age. At any age, however, the correlations indicate that the posterior teeth are more reliable indicators of age than anterior teeth.

The difference in dentin deposits among teeth of an individual may not be a general phenomenon among all odontocetes. Its occurrence is due to the closures of the pulp cavities of different teeth at different times. Some odontocetes such as *Physeter catodon* (Ohsumi et al. 1963), *Delphinapterus leucas* (Sergeant 1973), *Hyperoodon ampullatus* (Christensen 1973), *Phocoena phocoena* (Gaskin and Blair 1977) appear to continue deposition of dentin in all their teeth throughout life.

For *T. truncatus* and other small odontocetes which live longer than about 15 yr, however, a non-GLG method for age determination needs to be developed. Cemental examination would be useful if cementum was deposited continuously throughout life and at a constant rate as it is in humans (Zander and Hurzeler 1958). Kasuya (1976) has reported that cementum in *Stenella attenuata* is more reliable for estimating age than is the dentin, although no calibration for the upper ages was performed. Use of the rate constants for the racemization of aspartic acid in enamel (Helfman and Bada 1975) and dentin (Helfman and Bada 1976) also appears to be a promising approach.

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BEST, P. B. 1976. Tetracycline marking and the rate of growth layer formation in the teeth of a dolphin (*Lagenorhynchus obscurus*). S. Afr. J. Sci. 72: 216–218.

CHRISTENSEN, I. 1973. Age determination, age distribution and growth of bottlenose whales, *Hyperoodon ampullatus* (Forster), in the Labrador Sea. Norw. J. Zool. 21: 331–340.

CONOVER, W. J. 1971. Practical nonparametric statistics. John Wiley and Sons, New York, NY. 462 p.

GASKIN, D. E., AND B. A. BLAIR. 1977. Age determination of harbour porpoise, *Phocoena phocoena* (L.), in the western North Atlantic. Can. J. Zool. 55: 18–30.

HELFMAN, P. M., AND J. L. BADA. 1975. Aspartic acid racemization in tooth enamel from living humans. Proc. Natl. Acad. Sci. USA 72(8): 2891–2895.

1976. Aspartic acid racemization in dentine as a measure of ageing. Nature 262(5566): 279–281.

HEWER, H. R. 1964. The determination of age, sexual maturity, longevity, and a life-table in the grey seal (*Halichoerus grypus*). Proc. Zool. Soc. London 142(4): 593–624.

HUI, C. A. 1979. Correlates of maturity in the common dolphin, *Delphinus delphis*. Fish. Bull., U.S. 77(1): 295–300.

KASUYA, T. 1972. Growth and reproduction of *Stenella caeruleoalba* based on age determination by means of

dentinal growth layers. Sci. Rep. Whales Res. Inst. 24: 57–79.

1976. Reconsideration of life history parameters of the spotted and striped dolphins based on cemental layers. Sci. Rep. Whales Res. Inst. 28: 73–106.

LAWS, R. M. 1953. A new method of age determination of mammals with special reference to the elephant seal (*Mirounga Leonina*, Linn). Br. Antarct. Surv. Sci. Rep. 2: 11 p.

LOCKYER, C., A. BOYDE, AND W. F. PERRIN. 1980. Terminology. *In* Perrin and Myrick [ed.] Report of the workshop on age determination of odontocete cetaceans and sirenians September 1978. *In* Growth of odontocetes and sirenians: problems on age determination. Proc. of the international conference on determining age of odontocete cetaceans (and sirenians). International Whaling Commission. (In press)

MANSFIELD, A. W., AND H. D. FISHER. 1960. Age determination in the harbor seal, *Phoca vitulina* L. Nature 186(4718): 92–93.

OHSUMI, S., T. KASUYA, AND M. NISHIWAKI. 1962. Accumulation rate of dentinal growth layers in the maxillary tooth of the sperm whale. Sci. Rep. Whales Res. Inst. 17: 15–36.

PERRIN, W. F., AND A. C. MYRICK [ed.]. 1980. Report of the workshop on age determination of odontocete cetaceans and sirenians September 1978. *In* Growth of odontocetes and sirenians: problems on age determination. Proc. of the international conference on determining age of odontocete cetaceans (and sirenians). International Whaling Commission. (In press)

PERRIN, W. F., J. M. COE, AND J. R. ZWEIFEL. 1976. Growth and reproduction of the spotted porpoise, *Stenella attenuata*, in the offshore eastern tropical pacific. Fish. Bull., U.S. 74: 229–269.

RIDGWAY, S. H., R. F. GREEN, AND J. C. SWEENEY. 1975. Mandibular anesthesia and tooth extraction in the bottlenose dolphin. J. Wildl. Dis. 11: 415–18.

SERGEANT, D. E. 1959. Age determination of odontocete whales from dentinal growth layers. Norw. Whaling Gaz. (Nor. Hvalfangst-Tid.) 6: 273–288.

1962. The biology of the pilot whale or pothead whale *Globicephala malaena* (Traill) in Newfoundland waters. Bull. Fish. Res. Board Can. 132: 84 p.

1973. Biology of white whales (*Delphinapterus leucas*) in western Hudson Bay. J. Fish. Res. Board Can. 30: 1065–1090.

SERGEANT, D. E., D. K. CALDWELL, AND M. C. CALDWELL. 1973. Age growth and maturity of bottlenose dolphin (*Tursiops truncatus*) from northeast Florida. J. Fish. Res. Board Can. 30: 1009–1011.

ZANDER, H. A., AND B. HURZELER. 1958. Continuous cementum apposition. J. Dent. Res. 37: 1035–1044.

Estimation of Biological Power Functions from Group Measurements[1,2]

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CAMMEN, L. M. 1980. Estimation of biological power functions from group measurements. Can. J. Fish. Aquat. Sci. 37: 716–719.

Nonlinear regression can be used to estimate the parameters of power functions ($Y = \alpha X^{\beta}$) when the only available data are group measurements of the dependent variable and individual measurements of the independent variable. Possible applications are to length–weight and weight–metabolism relations of natural populations of organisms.

Key words: power functions, length–weight relations, metabolism–weight relations, nonlinear regression

CAMMEN, L. M. 1980. Estimation of biological power functions from group measurements. Can. J. Fish. Aquat. Sci. 37: 716–719.

On peut utiliser une régression non linéaire pour estimer les paramètres de fonctions exponentielles ($Y = \alpha X^{\beta}$) dans les cas où les seules données disponibles sont des mensurations par groupes de la variable dépendante et mensurations individuelles de la variable indépendante. La régression peut être appliquée aux relations longueur–poids et poids–métabolisme de populations naturelles d'organismes.

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ESTIMATION of the parameters of power functions of the form

$$Y = \alpha X^{\beta}.$$

is a common problem in many areas of biology. Power functions have been used to model the relation between a variety of variables including length and weight, weight and respiration, and weight and feeding. Although the parameters have been estimated by a variety of methods — least-squares linear regression on log-transformed data, functional (geometric mean) regression (Ricker 1973), and nonlinear regression (Pienaar and Thomson 1969; Matthews and Hestad 1977) — a common feature of these methods is that they all have required the measurement of both independent and dependent variables for a number of individuals. The purpose of this paper is to point out that the parameters of the power function may also be estimated with group measurements of the dependent variable alone as long as individual measurements are available for the independent variables.

Method — Consider a population with two measurable attributes (for example weight, W, and length, L) whose relation can be described by a power function,

$$(1)\quad W = \alpha L^{\beta}.$$

The usual problem is to estimate α and β from repeated observations of individuals drawn from the population. In that case the parameters can be estimated from the logarithmic transformation of eq. 1 using least-squares linear regression,

$$(2)\quad \text{Log } W = \text{Log } \alpha + \beta \text{ Log } L.$$

Alternatively, α and β can be estimated using nonlinear regression without transformation of eq. 1. The choice between these two methods should depend on the assumed distribution of error within the model. If the errors are additive and relatively constant, then the total distribution is generally normal and the nonlinear technique is preferable. On the other hand, if the error terms are multiplicative, then the distribution will usually be lognormal and the logarithmic transformation restores normality and homogeneity of variance. Ricker (1973) has suggested that a functional (geometric mean) regression is preferable to least-squares regression for most regression situations in fishery research. Several authors have discussed the effects of various forms of weighting the regression to achieve the best possible estimates of the parameters (Pienaar and Thomson 1969; Matthews and Hestad 1977; Silvert 1979; Smith 1979).

In this paper I examine the situation where individual measurements of the independent variable (length) are available but only group measurements of the dependent variable (weight) exist. If W_i is the total weight for a group of N individuals, each with weight w_{ij} and length L_{ij}, then

$$(3)\quad W_i = w_{i1} + w_{i2} + \ldots + w_{iN}$$

or

$$(4)\quad W_i = \alpha L_{i1}^{\beta} + \alpha L_{i2}^{\beta} + \ldots + \alpha L_{iN}^{\beta} = \alpha \sum_{j=1}^{N} L_{ij}^{\beta}.$$

If there is a series of M observations of group weights and individual lengths, then

$$(5)\quad W_1 = \alpha \sum_{1}^{N} L_{1j}^{\beta}$$

$$W_2 = \alpha \sum_{1}^{N} L_{2j}^{\beta}$$

$$\vdots$$

$$W_M = \alpha \sum_{1}^{N} L_{Mj}^{\beta};$$

of course, the number of individuals within each group may vary. In this case we can apply nonlinear regression techniques to eq. 5 to estimate α and β with W_i as the dependent variable and the L_{ij} as independent variables.

It is not my intention to consider here the techniques of nonlinear regression since the subject is discussed in most statistical textbooks. Other authors have discussed these methods as they apply to marine biology (Pienaar and Thomson 1969; Matthews and Hestad 1977; Silvert 1979; Smith 1979) and there are several computer statistical packages such as BMDP (Dixon 1975) with nonlinear regression procedures which allow the user to specify his or her own function (eq. 5 in this case). In addition, the BMDP procedure allows the regression to be weighted by a user-specified variable. Smith (1979) has discussed techniques for approximating confidence intervals for the parameters of nonlinear models.

Comparison with other techniques — To illustrate the use of this technique and to compare it with other techniques of parameter estimation for power functions, I developed a simulated fish population. The individuals of this population had lengths (L) between 100 and 600 mm and weights (W, in grams) generated according to

$$(6)\quad W = 5 \times 10^{-5} L^{3}.$$

Twenty random samples of 100 fish each were drawn from this population. Variability was then introduced to simulate either a normal or a lognormal distribution of error in the weights. For the first 10 samples, random numbers were drawn from a normal distribution ($\bar{x} = 1.0$, $\sigma = 0.3$) and multiplied by the individual weights, approximating a normal distribution of error. For the second group of 10 samples, random numbers were drawn from a separate normal distribution ($\bar{x} = 0$, $\sigma = 0.3$) and added to the logarithms of the individual weights which were then transformed by taking the antilogarithms; this procedure resulted in an approximately lognormal distribution of error.

Four techniques were used to estimate the parameters of eq. 6 for each of the 20 sets of 100 individual "observations" (Tables 1, 2):

1) Nonlinear regression on the original data (using BMDP3R);
2) Weighted nonlinear regression on the original data with the weighting factors inversely pro-

TABLE 1. Comparison of various methods of estimating the parameters of the function $W = \alpha L^{\beta}$ where W is the weight and L the length of a fish from a simulated population with a normal error distribution. Separate estimates of the parameters and the goodness-of-fit for each of the 10 sets of 100 fish were averaged to give means ± standard deviation. The original parameters of the model used to create the population, before random variation was introduced, are also given.

Method	$\alpha(\times 10^{-5})$	β	r^2
1) Nonlinear regression on the individual data	26.718±51.627	2.956±0.328	0.846±0.042
2) Weighted nonlinear regression on the individual data	6.632±5.860	2.996±0.174	0.820±0.060
3) Least-squares linear regression on log-transformed individual data	5.068±1.963	2.999±0.072	0.837±0.044
4) Functional (GM) regression on log-transformed individual data	2.976±1.294	3.095±0.083	0.838±0.046
5) Nonlinear regression on grouped data	58.434±128.311	3.090±0.489	0.839±0.045
6) Weighted nonlinear regression on grouped data	26.280±49.868	3.041±0.392	0.843±0.044
Underlying function	5	3	0.836±0.052

TABLE 2. Comparison of various methods of estimating the parameters of the function $W = \alpha L^{\beta}$ where W is the weight and L the length of a fish from a simulated population with a lognormal error distribution. Separate estimates of the parameters and the goodness-of-fit for each of the 10 sets of 100 fish were averaged to give means ± standard deviation. The original parameters of the model used to create the population, before random variation was introduced, are also given.

Method	$\alpha(\times 10^{-5})$	β	r^2
1) Nonlinear regression on the individual data	6.013±6.935	3.107±0.236	0.863±0.031
2) Weighted nonlinear regression on the individual data	4.360±2.756	3.047±0.111	0.850±0.036
3) Least-squares linear regression on log-transformed individual data	5.108±1.760	3.005±0.056	0.857±0.031
4) Functional (GM) regression on log-transformed individual data	3.620±1.168	3.064±0.052	0.860±0.031
5) Nonlinear regression on grouped data	81.023±135.972	3.042±0.654	0.851±0.032
6) Weighted nonlinear regression on grouped data	29.925±48.818	2.956±0.330	0.858±0.034
Underlying function	5	3	0.854±0.031

portional to the weights of the fish (using BMDP3R);

3) Least-squares linear regression on the log-transformed data;
4) Functional (GM) regression on the log-transformed data.

Each set of 100 observations was then divided into groups of random size (but with a maximum of 10 observations per group); a total of 20 groups resulted. The total weight was calculated for each group from the sum of the individual weights and then the data were treated as though only this group weight and the individual lengths were available. Two more estimates of the power function were then calculated for each of the 20 sets of grouped data using eq. 5 and the following techniques (Tables 1, 2):

5) Nonlinear regression (using BMDP3R);
6) Weighted nonlinear regression with the weighting factors inversely proportional to the total weight of each group (using BMDP3R).

The power functions estimated with these six techniques, including those using the grouped data, fit the original data equally well regardless of whether the underlying error distribution was normal or lognormal (Tables 1, 2). In addition, the fit of these curves was essentially the same as the fit of the function originally used to generate the data, eq. 6. The techniques using the grouped data (5 and 6) came close to the true value for β, but did not give reliable estimates for α; however, the use of weighting generally improved both the accuracy and precision of the parameter estimates. Whether the error distribution was normal or lognormal had little effect on the accuracy or precision of the estimates for the grouped data.

Discussion — Nonlinear regression has been shown to be a useful way to fit power functions to sets of data with grouped observations of the dependent variable, data that otherwise could not be analyzed. The goodness-of-fit values for curves generated with this method were similar to those for curves generated from complete individual data. In addition, the estimates of the exponents of the power functions, which are normally the biologically significant parameters (see for example Ricker 1973), were relatively unbiased as well as showing little effect of the underlying error distribution. It appeared from the examples given here that a weighting procedure is desirable during the regression analysis. Further analysis may indicate the exact nature of the variance of the estimated parameters, but until such an analysis has been carried out the techniques in Smith (1979) can be applied to approximate the variances.

Some possible applications of this technique might include:

1) Reduction of processing time for the determination of length–weight relations. Although conventional regression analyses may be applicable, it should be noted that use of the technique given here will approximately halve the number of measurements to be taken for each sample (only lengths need to be meas-

ured for each individual). Because most of the time required for each sample is frequently due to processing and not to collection, a reduction in the processing time would allow collection of a greater number of samples.

2) Determination of width–weight relations on animals such as polychaetes which fragment during collection. With the widths of the heads, for example, and the total weights of the samples, the appropriate relation could be estimated even though no intact individuals were ever collected.

3) Determination of various weight–metabolism relations for natural populations. With this technique it would not be necessary to carry out measurements of excretion, feeding rate, or respiration on a collection of animals of similar size to standardize the results. Natural collections of various-sized individuals could be used to give measures of group metabolism and the results could then be expressed as a power function relating individual metabolism to individual size. This technique could reduce the amount of manipulation required because it would not be necessary to separate groups of similar-sized individuals prior to the experiments.

This technique will be applicable to many functions other than power functions. In general, the parameters of a function where one of the variables may be meaningfully measured for a group as well as for an individual can be estimated by applying nonlinear regression techniques to the appropriate reexpression of the function.

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DIXON, W. J. 1975. BMDP: biomedical computing programs. University of California Press, Berkeley, CA.

MATTHEWS, J. B. L., AND L. HESTAD. 1977. Ecological studies on the deep-water pelagic community of Korsfjorden, western Norway. Length/weight relationships for some macroplanktonic organisms. Sarsia 63: 57–63.

PIENAAR, L. V., AND J. A. THOMSON. 1969. Allometric weight–length regression model. J. Fish. Res. Board Can. 26: 123–131.

RICKER, W. E. 1973. Linear regressions in fishery research. J. Fish. Res. Board Can. 30: 409–434.

SILVERT, W. 1979. Practical curve fitting. Limnol. Oceanogr. 24: 767–773.

SMITH, W. R. 1979. Parameter estimation in nonlinear models of biological systems. Fish. Mar. Serv. Tech. Rep. 889: 90 p.

Detection of *Corynebacterium salmoninus* by Direct Fluorescent Antibody Test

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A direct fluorescent antibody test (FAT) was developed for detecting *Corynebacterium salmoninus*, causative agent of bacterial kidney disease. The direct procedure was found to be as specific as the previously described indirect test, and requires only 7 min—about $\frac{1}{10}$ as much time—to perform. A comparison of the direct FAT with the Gram stain for sensitivity of detecting the bacterium indicated the fluorescent procedure to be 100 to 1000 times more sensitive. Furthermore, the FAT is highly effective and efficient for screening large numbers of samples.

Key words: Corynebacterium salmoninus, detection, bacterial kidney disease, direct fluorescent antibody test

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Un essai d'anticorps par fluorescence directe (FAT, « fluorescent antibody test ») fut mis au point pour détecter *Corynebacterium salmoninus*, agent responsable de la maladie bactérienne du rein. On constate que la méthode directe est aussi spécifique que l'essai indirect déjà décrit et ne requiert que 7 min, soit $\frac{1}{20}$ du temps. Une comparaison du FAT direct avec le colorant de Gram quant à sa sensibilité à détecter la bactérie indique que la méthode fluorescente est de 100 à 1000 fois plus sensible. En outre, le FAT permet de séparer plus efficacement de grandes quantités d'échantillons.

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SEROLOGICAL procedures are being used increasingly for diagnosis of infectious diseases of fishes. *Corynebacterium salmoninus*, the cause of kidney disease (KD) of salmonid fishes (Sanders and Fryer 1978), grows slowly and is difficult to isolate. Accordingly, serological techniques are particularly useful in diagnosis of this disease. Bullock et al. (1974) described an agglutination test, and Chen et al. (1974) and Kimura et al. (1978) reported an immunodiffusion procedure for diagnosing KD in infected tissues. Bullock and Stuckey (1975) described an indirect fluorescent antibody test (FAT) which, though effective, requires 2 to 2½ h to perform, and is therefore not practical for screening large numbers of samples. We describe a direct FAT that requires only 7 min to perform. The test is appropriate for diagnosis of KD and is especially useful for detecting subclinical infections.

Materials and methods — Fluorescein isothiocyanate (FITC) was conjugated with the purified IgG fraction of rabbit or goat anti-*C. salmoninus* serum (Bullock and Stuckey 1975) using standard techniques (Johnson and Holborow 1973). Conjugates were also prepared from IgG fractions of goat and rabbit sera taken before immunization.

The following procedures were used to standardize and test the conjugate. Test material was placed on slides, air-dried, and fixed at 60°C for 2 min. Conjugate, containing a 1:150–1:200 dilution of rhodamine (Difco) counterstain, was added and allowed to react for 5 min at 20–25°C. Slides were then rinsed, washed 2 min in phosphate-buffered saline (PBS) at pH 7.2, and air-dried. A drop of pH 9.0 mounting fluid (Difco) was placed on test areas, a coverslip added, and the material examined under oil immersion. We used an AO Spencer incident light fluorescence microscope with a mercury vapor lamp, BG-12 exciter filter, 500-nm dichroic beam splitter, and 515-nm barrier filter. Alternatively, a tungsten halogen lamp was used, and an FITC (515 KP) filter replaced the BG-12 exciter filter.

Optimal dilution of the goat or rabbit conjugates was determined by testing doubling dilutions in PBS starting with 1:10. Samples of formalin-killed *C. salmoninus* cells were treated with each dilution of conjugate and examined for fluorescence intensity. The degree of fluorescence was judged on a scale of 0 to +4, with +4 being highest intensity (Jones et al. 1978). A working concentration was established to be the second-to-highest dilution giving +4 fluorescence, which proved to be 1:80 for goat conjugate and 1:40 for rabbit conjugate.

Direct specific and preimmune conjugates were applied in the direct FAT to the same *C. salmoninus* cultures, Gram-negative fish pathogens, and fast-growing Gram-positive cultures from fish that were used for evaluating the indirect *C. salmoninus* FAT (Bullock and Stuckey 1975). Kidney tissues from chinook salmon (*Oncorhynchus tshawytscha*) with KD were also used in the evaluation. The medium of Evelyn (1977) was used to isolate *C. salmoninus* from chinook salmon kidney tissues. The direct procedure was also compared with the Gram stain for detecting *C. salmoninus* cells in infected kidney tissues. The number of bacteria in heavily infected kidney tissue was determined by direct microscopic count, using a Petroff–Hauser counting chamber. Serial 10-fold dilutions were prepared from the ground, infected tissues, using normal kidney tissues as diluent. Comparison of each 0.01-mL dilution was examined after Gram staining or direct FAT. Enumeration of the cells in a known volume enabled us to calculate the number of *C. salmoninus* organisms in test smears. The sensitivity of Gram staining and direct FAT was also compared by determining the incidence of KD among 130 dead trout from a research laboratory where KD was known to be present. Records show that the incidence of KD among trout at the laboratory was low and KD was not suspected to be the cause of death of the 130 trout.

Finally, the usefulness of the direct FAT in screening samples for KD bacteria was determined at two different laboratories by using 180 kidney and fecal samples from populations of feral brook (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*) known to include carriers.

Results — The direct FAT with either goat or rabbit conjugate was specific for *C. salmoninus*. Cultures of the KD bacterium and KD-infected chinook salmon kidney tissue had a +4 fluorescence. The FAT identification of *C. salmoninus* was verified by isolating the bacterium from infected kidney tissue and subsequent agglutination of isolates with *C. salmoninus* antiserum. None of the Gram-negative cultures nor the fast-growing Gram-positive cultures isolated from fish showed fluorescence. All cultures were negative with a 1:10 dilution of conjugate prepared from preimmune sera.

The direct FAT was more sensitive than Gram staining in comparative tests for sensitivity of detection. Cells of *C. salmoninus* diluted in normal kidney homogenates could be detected by direct FAT if there were at least 10^4 cells per smear. In contrast, a minimum of 10^6 to 10^7 organisms were necessary for detection by Gram staining. Sixty-eight percent of the

130 kidney samples from dead fish were positive with FAT but only 35% with the Gram stain. The FAT was clearly the superior method.

The examination of fecal samples from the feral brook trout by the two laboratories revealed a low and similar incidence of infection. In one laboratory, 15 fecal samples had 2 to 20 fluorescent cells per specimen. The second laboratory reported 11 fecal samples with fluorescent cells. There was agreement on six of the samples which were scored as positive by both laboratories. Although KD cells were found in feces, none of the kidney tissues analyzed by either laboratory were positive. Some fecal samples had fluorescing bacteria two to three times larger than *C. salmoninus*, but were easily distinguishable from the KD bacterium.

Discussion — The direct FAT for *C. salmoninus* proved to be as specific and sensitive for identifying the organism in clinical KD and for detecting subclinical cases as the indirect procedure. However, the direct test requires only 7 min and is far more practical than the indirect test for screening large numbers of samples. The superiority of the direct FAT in detecting KD bacteria was evident from quantitative studies showing that 100 times more bacteria were needed for detection by Gram staining. In addition, almost twice as many kidney samples from the dead fish were shown to be positive by FAT. This relation between the two methods agrees with results of Franěk et al. (1971) in the detection of *Fransiella tularensis*.

The sensitivity of FAT and ease of application of the direct procedure have improved detection of subclinical KD. The reliability of the direct test in detecting low-level infections demonstrated the general agreement of results obtained at the two laboratories.

Our finding that fecal samples were superior to kidney samples for detecting subclinical infection with *C. salmoninus* also agrees with the results of Lobb (1976). Fecal samples offer a nondestructive means for screening populations for KD. Moreover, sensitivity could be increased if procedures were developed for concentrating *C. salmoninus* cells in fecal material.

The presence of an FAT-positive bacterium, other than *C. salmoninus*, in some of the fecal samples is not considered to be a barrier to the use of the direct FAT for diagnosis of KD. The cross-reactivity has been observed only in connection with a very characteristic bacterial cell type and, although it has not been identified, it is clearly distinguishable from *C. salmoninus*.

The direct FAT conjugate was being used at 16 laboratories by mid-1979 and the results indicated the procedure to be sound.

Qualified users may obtain samples of the standardized lyophilized conjugate from The Biologics Section of The National Fish Health Research Laboratory.

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BULLOCK, G. L., AND H. M. STUCKEY. 1975. Fluorescent antibody identification and detection of the *Corynebacterium* causing kidney disease of salmonids. J. Fish. Res. Board Can. 32: 2224–2227.

BULLOCK, G. L., H. M. STUCKEY, AND P. K. CHEN. 1974. Corynebacterial kidney disease of salmonids: growth and serological studies on the causative bacterium. Appl. Microbiol. 28(5): 811–814.

CHEN, P. K., G. L. BULLOCK, H. M. STUCKEY, AND A. C. BULLOCK. 1974. Serological diagnosis of corynebacterial kidney disease of salmonids. J. Fish. Res. Board Can. 31(12): 1939–1940.

EVELYN, T. P. T. 1977. An improved growth medium for the kidney disease bacterium and some notes on using the medium. Bull. Off. Int. Epizoot. 87(5–6): 511–513.

FRANĚK, J., V. KUBIN, AND O. PROCHAZKA. 1971. The use of immunofluorescence in grouping of streptococci and in diagnosis of tularemia. Ann. N.Y. Acad. Sci. 177: 12–22.

JOHNSON, G. D., AND E. J. HOLBOROW. 1973. Immunofluorescence, p. 18.1–18.20. *In* D. M. Weir [ed.] Handbook of experimental immunology. Vol. 1, Immunochemistry. 2nd ed. Blackwell Scientific Publications, Oxford, London, Edinburgh, Melbourne.

JONES, G. L., G. A. HEBERT, AND W. B. CHERRY. 1978. Fluorescent antibody techniques and bacterial applications. U.S. Department of Health, Education, and Welfare Publication Number (CDC) 78-8364. 118 p.

KIMURA, T., Y. EZURA, K. TAJIMA, AND M. YOSHIMIZU. 1978. Serological diagnosis of bacterial kidney disease (BKD); immunodiffusion test by heat stable antigen extracted from infected kidney. Fish Pathol. 13(2): 103–108.

LOBB, C. J. 1976. Salmonid kidney disease: diagnosis by immunodiffusion. M.S. thesis, Utah State University, Logan, Utah. 58 p.

SANDERS, J. E., AND J. L. FRYER. 1978. *Corynebacterium salmoninus* sp. nov. the causative agent of bacterial kidney disease; selected biochemical properties and pathogenesis in salmonid fishes, p. 28–33. Proc. of the Third Biennial Fish Health Section of the American Fisheries Society and Ninth Annual Midwest Fish Disease Workshops at Kansas City, Missouri, August 15–18, 1978.

BIONESS, a New Design in Multiple Net Zooplankton Samplers

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BIONESS (Bedford Institute of Oceanography Net and Environmental Sampling System), a new multiple-net zooplankton and micronekton sampler, uses a new design concept with the nets arranged horizontally rather than vertically as in earlier multiple-net samplers. Ten 1.0-m² nets open sequentially and are towed at 3 knots on a conductor cable that transmits and receives information between the ship and sampler. On the sampler are sensors for temperature, conductivity, depth, illumination, pitch, roll and yaw, and net closure together with internal and external flowmeters and a 35-mm camera equipped with a strobe light. This system can collect biological samples and environmental data simultaneously and photograph animals in front of the sampler.

Key words: samplers, zooplankton, micronekton, instrumentation, photography, data collection, design

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Le BIONESS (« Bedford Institute of Oceanography Net and Environmental Sampling System »), un échantillonneur de zooplancton et de micronecton à filets multiples, fait appel à un concept nouveau selon lequel les filets sont disposés horizontalement plutôt que verticalement comme les échantillonneurs précédents à filets multiples. Le système comprend 10 filets de 1,0 m² qui s'ouvrent successivement et sont traînés à une vitesse de 3 noeuds sur cable conducteur qui transmet et reçoit l'information entre le bateau et l'échantillonneur. Des détecteurs de température, conductivité, profondeur, illumination, tangage, roulis et lacet sont fixés sur l'échantillonneur, de même qu'un mécanisme de fermeture des filets, courantomètres interne et externe, et caméra 35 mm équipée d'une lumière stroboscopique. Grâce à ce système, il est possible de recueillir des échantillons biologiques en même temps que des données sur l'environnement et photographier des animaux en avant de l'échantillonneur.

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MULTIPLE net samplers described by Wiebe et al. (1976) and Sameoto et al. (1977) had the nets and their closing bars stacked vertically making the front profile much larger than the mouth opening of a single net of the sampler. Our aim was to eliminate the highly visible structures of the frame and make the front view inconspicuous and streamlined to reduce the visibility and pressure wave of the sampler as it moved through the water. This note gives a brief description of the new sampler and its operation. Sameoto et al. (1979) gives a complete description of the construction and operation of the sampler.

Construction and operating principles — The sampler frame resembles an open-ended steel box, 146 cm high, 146 cm wide, and 74 cm deep (Fig. 1). Ten nets are attached to 10 horizontal bars (steel channel) stacked one behind the other. (See Fig. 2 where net No. 1 is in the open position). Figure 2 also shows the attachment of nets to the horizontal bars. The lower lip of the net is attached to the bar in front of the net and the upper lip attached to the horizontal bar behind the first bar. Therefore, as a horizontal bar is released (i.e. No. 1, Fig. 2), one net is closed as the next net behind it is opened. Aligning the bars one behind the other minimizes the cross-sectional area of the sampler. Each horizontal bar is lead-filled (21 kg) to facilitate rapid closing of the net as the bar descends between two vertical runners (steel tubes). Teflon wheels and pads reduce friction. These are necessary since there is an estimated 100–200 kg of horizontal force on the net bar during a tow. When the sampler is loaded the horizontal bars are attached to a release mechanism (see Sameoto et al. 1977, 1979) by steel wires which hold the bars up at the top of the frame.

The first net is open when the sampler is put in the water because the bottom lip of the net is attached to the lower crosspiece of the frame. The stability of the sampler does not depend on a net being open since the two side plates (Fig. 1) act as rudders and prevent yawing, as well as adding strength and rigidity to the

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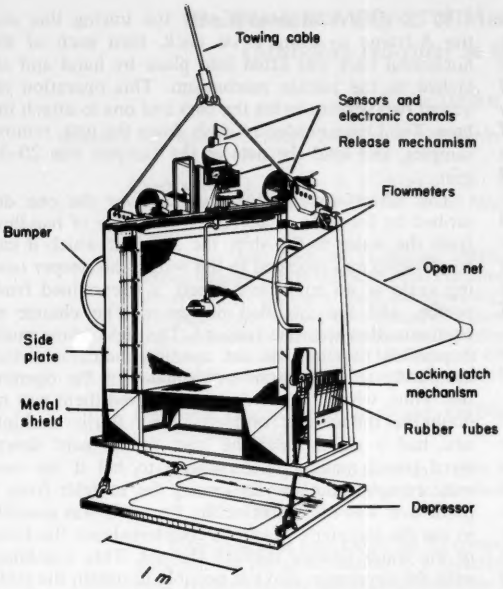

FIG. 1. Diagrammatic representation of BIONESS as it would look being towed with the first net open. The conductor cables from the towing line to the various pressure cases have been omitted for simplicity sake.

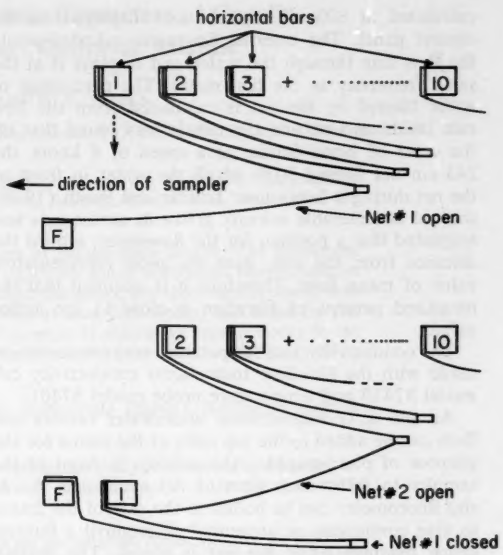

FIG. 2. Diagrammatic representation of horizontal bars showing net arrangement as sampler is first put in the water, and net arrangement after net No. 2 is opened.

frame. The sides of the net are attached to the vertical runners by five lines attached to steel rings that slide freely on the runners. These keep the sides of the net tight when open and allow them to fold when the net is closed. The arrangement of the horizontal bars prevents contamination of the nets before and after closing since they are not in the direction of the flow. This is an advantage over the previous samplers where the mouths of the closed nets are aligned in the flow direction and could be reopened by strong water pressures. The folds of the sides of the nets are protected from contamination before opening by Velcro strips sewn in the mouths of the net that keep the folds tightly closed. The side folds are further protected from contamination by a metal shield (Fig. 1) attached to the bottom corners of the frame that deflect animals that might otherwise enter the folds of the closed nets.

The depressor (Fig. 1) is similar in design and size (153 × 102 cm) to that described by Sameoto et al. (1977). New force release doors in the center of the depressor are held closed by torsion springs and open downward when the force of the entire depressor reaches 900 kg. This acts as a safety release to ensure that the depressor forces in rough seas do not exceed the breaking strength of the towing cable. In addition, a weak link is incorporated in one line to the depressor and breaks when the depressor force exceeds 1800 kg.

The vertical angle of the sampler mouth can be changed by altering the position of the towing cable and the depressor. The optimum angle and speed are 10° and 3 knots, which gives the fastest closing time (2–4 s) for the net after the close command is given. At 0° angle the closing time is slightly longer (6–8 s).

The net counter is located at the bottom of the frame. As each horizontal bar reaches the bottom of the frame, it strikes 1 of the 10 pins on a rotating shaft and an electrical switch indicates net closure. The horizontal bar is locked firmly in place by a pair of mechanical latches (Fig. 1). There are 10 independent latches on the shaft on each side of the frame.

The net is towed with an 8-conductor, 9.53-mm-diameter cable, breaking strength 5443 kg. The sampler weighs 782 kg and is best handled from an A-frame or large crane on the ship's stern. It can be handled with a crane on the forward part of the ship and towed from the side, but this limits operations to calm and moderate seas due to the ship's roll.

Sensors and electronics — There are two electronic flowmeters, one in the mouth one third the distance from the sides, and one protruding from the outside of the frame (Fig. 1). The internal meter moves back on a track as each net falls and therefore is in the same position for all 10 nets. The internal meter data are combined with information on the pitch, roll and yaw of the sampler and fed into a microprocessor (described later) which calculates total volume of water passing through the mouth of the net. This value is

calculated at 800-ms intervals and displayed on the control panel. The external flowmeter calculates only the flow rate through the water and displays it at the same frequency as the first meter. The percentage of water filtered by the net is calculated from the flow rate inside and outside the net. It was found that off the coast of Nova Scotia, at a speed of 4 knots, the 243-μm net filtered 95% of all the water in front of the net during a 5-min tow. Tranter and Smith (1968) showed considerable velocity gradients across nets and suggested that a position for the flowmeter, a third the distance from the side, gave the most representative value of mean flow. Therefore it is assumed that the measured percent of filtration is close to the actual value.

The conductivity and temperature measurements are made with the Guidline Instruments conductivity cell model 87410 and temperature probe model 87401.

An EG & G International underwater camera and flash can be added to the top sides of the frame for the purpose of photographing the animals in front of the sampler to detect any signs of net avoidance. An in situ fluorometer can be bolted to the side of the frame to give continuous or sequential chlorophyll *a* fluorescence readings while the net is towed. The surface electronics are controlled by a microcomputer which processes data coming from the net display or recording on magnetic and/or paper tape.

Sampler performance — The towing wire and depressor attachments could be adjusted so that at any towing speed up to 4 knots the mouth angle would be 0° from the vertical. Roll and yaw were under ±5° once the bridles of the depressor were set properly. The towing cable tension at speeds from 2–6 knots ranged between 990 and 1600 kg. Sampler depth varied only ±0.2 m when towed in seas with 1-m waves.

The mouth area to surface area of the nets had a ratio of 1:10. Two mesh sizes were used, 243 and 1000 μm. When the coarse mesh net was opened after the fine mesh net, there was no noticeable change in sampler angle or depth. The nets were dyed dark green or blue and all surfaces of the sampler were painted flat grey to prevent light reflection. Catches in the 243-μm nets had animals ranging from small copepods and euphausiids to squid up to 15 cm, as well as an array of pelagic fish including anchovy and capelin ranging up to 20 cm long. There was no noticeable difference in the maximum size of animals caught in the two different mesh-sized nets. Fish were never caught in the sampler described by Sameoto et al. (1977), even though sonar indicated that capelin were abundant at the towing depth.

Despite the weight of BIONESS, it was easier to handle than the sampler described by Sameoto et al. (1977). BIONESS needed only the towing line and the A-frame to bring it on deck, then each of the horizontal bars was lifted into place by hand and attached to the release mechanism. This operation required two persons to lift the bars and one to attach the bars. Total time needed to wash down the nets, remove samples, and load the nets of the sampler was 20–30 min.

The advantages of this sampler over the one described by Sameoto et al. (1977) are ease of handling from the water to the ship, the speed at which it can be reloaded and returned to the water, the steeper towing angle at an equivalent speed, a streamlined front profile, and the modified depressor. The chance of contamination was also reduced. The rigid frame made it possible to align the net opening indicator switch accurately at the bottom of the path of the opening net. Thus, when the switch was triggered there was no doubt that the net was completely open. Earlier multiple nets had a switch near the start of the nets' downward travel making it impossible to tell if the nets were completely open. By towing the sampler from a point that was off center of the frame, it was possible to use the sampler's weight to counterbalance the force of the water flowing through the net. This, combined with the depressor, make it possible to obtain the steep vertical mouth angle when towing. The vertical dimension of the sampler made it possible to use BIONESS off small boats in the range of 20-m length if a 3-m A-frame was available. An additional advantage as a result of the box frame was the ease at which various environmental sensors could be added without increasing the vertical dimension or making it more visible to animals.

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SAMEOTO, D. D., L. O. JAROSZYNSKI, AND W. B. FRASER. 1977. A multiple opening and closing plankton sampler based on the MOCNESS and N.I.O. nets. J. Fish. Res. Board Can. 34: 1230–1235.

1979. Bedford Institute of Oceanography net and environmental sensing system (BIONESS) construction details. Fish. Mar. Serv. Tech. Rep. 903: 47 p.

TRANTER, D. J., AND P. E. SMITH. 1968. Filtration performance, p. 27–56. *In* Zooplankton sampling. Paris, UNESCO. Monogr. Oceanogr. Methodol. 2.

WIEBE, P. H., K. H. GURT, S. H. BOYD, AND A. W. MORTON. 1976. A multiple opening/closing net and environmental sensing system for sampling zooplankton. J. Mar. Res. 34: 313–326.

Multivariate Analysis of Meristic Characters of Capelin (*Mallotus villosus*) in the Northwest Atlantic

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CARSCADDEN, J. E., AND R. K. MISRA. 1980. Multivariate analysis of meristic characters of capelin (*Mallotus villosus*) in the Northwest Atlantic. Can. J. Fish. Aquat. Sci. 37: 725–729.

Counts were made on 11 meristic characters of mature male capelin. The Bonferroni method identified three characters that exhibited significant annual differences. Interpopulation differences were compared using the remaining eight characters, and three capelin stocks were identified. Other studies have tentatively identified at least four stocks, and based on this analysis, meristic characters show only limited promise in separating capelin stocks in the Northwest Atlantic. However, the sequence of statistical procedures used in this study offers potential in other stock discrimination studies.

Key words: capelin, *Mallotus villosus*; meristics, multivariate analyses, Bonferroni method, discriminant function

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Onze caractères méristiques ont été comptés sur des capelans mâles matures. La méthode de Bonferroni permet d'identifier trois caractères qui varient de manière significative annuellement. Utilisant les huit autres caractères, nous avons comparé les différences entre populations et identifié trois stocks de capelans. D'autres études ont conduit à l'identification provisoire d'au moins quatre stocks. Cette analyse démontre que les caractrères méristiques sont d'une utilité limitée dans la séparation des stocks de capelans dans l'Atlantique nord-ouest. Cependant, la suite d'opérations statistiques utilisées dans cette étude offre des possibilités pour d'autres études de différentiation de stocks.

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IN spite of the fact that capelin (*Mallotus villosus*) is the most important fish fodder resource in the Northwest Atlantic (Bailey et al. 1977; Winters and Carscadden 1978) and a large commercial capelin fishery has recently developed, firm evidence for the existence of stocks of capelin does not exist.

Analysis of vertebral counts (Templeman 1948; Pitt 1958) and electrophoretic analysis of liver and skeletal muscle esterases (Payne 1975, 1976) were unsuccessful in discriminating stocks of capelin in the Newfoundland area. Based on known seasonal distribution patterns and growth differences, Campbell and Winters (1973) and Winters (1974) suggested four tentative stock divisions for capelin in the Canadian Atlantic; Labrador–Northeast Newfoundland stock, Northern Grand Bank–Avalon stock, South Grand Bank stock, and St. Pierre–Green Bank stock. Sharp et al. (1978) suggested that meristic characters offered little potential for capelin stock differentiation but that morphometric characters showed some promise. However, their study was primarily concerned with capelin from the Gulf of St. Lawrence and only two locations were sampled in the Newfoundland area.

Considerable impetus was given to the present study because of the tentative nature of the suggested stock divisions and the trends in the capelin fishery. Catches rose rapidly after 1972 but declined in the southern area (ICNAF Div. 3LNOP) in 1977 and over most of the ICNAF area (ICNAF Div. 2J, 3KLNOP) in 1978. This decline in catch was especially evident in Div. 3N (South Grand Bank Stock of Winters (1974)) and Div. 3L (Northern Grand Bank–Avalon stock of Winters (1974)). In addition, spawning capelin were not abundant on beaches on the south coast of Newfoundland (St. Pierre–Green Bank stock of Winters (1974)) in 1978 and 1979.

The purposes of this paper are to present the results of a multivariate analysis of a large number of meristic characters of capelin from the Gulf of St. Lawrence and Newfoundland and to illustrate the use and sequence of statistical procedures which may be potentially useful in other studies of this type.

Collection and treatment of specimens — The tentative stock divisions suggested by Campbell and Winters

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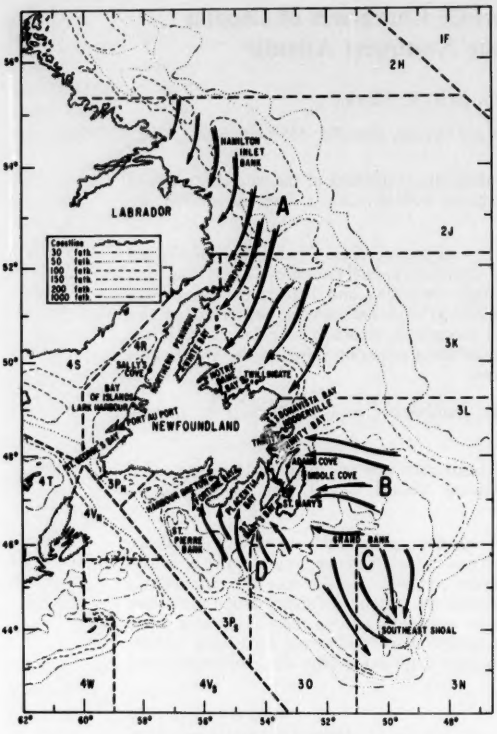

FIG. 1. Location of sampling sites for present capelin meristics study and tentative capelin stocks defined by Campbell and Winters (1973) and Winters (1974).

(1973) were used as a basis for sample collections and later statistical analysis. In all, 12 sampling sites were chosen (Fig. 1). All specimens were mature males and were frozen prior to analysis.

The following meristic counts were made: left pectoral fin rays, dorsal fin rays, anal fin rays, pyloric caecae, branchiostegals, upper gill rakers, lower gill rakers, dorsal secondary caudal rays, ventral secondary caudal rays, precaudal vertebrae, and caudal vertebrae according to conventions described by Sharp et al. (1978). All counts were made after cleaning and staining (Taylor 1967) with the exception of the counts of pyloric caecae which were made prior to preservation in formalin. Ages were determined from otoliths removed prior to cleaning and staining. Two ages, 3 and 4 yr (1972 and 1973 year-classes), were abundant in the samples and only fish of these year-classes, randomly selected from the total sample, were retained to give meristic counts.

In addition to the four stocks (A, B, C, D, in Fig. 1) suggested by Campbell and Winters (1973), we grouped samples from the western coast of Newfoundland bordering on the Gulf of St. Lawrence into a fifth stock (E).

Evidence from Soviet (Kovalyov and Kudrin 1973), Canadian (Campbell and Winters 1973), and Norwegian (Dragesund and Monstad 1973) surveys suggest that mature capelin occurring in ICNAF Div. 3L in early spring are a mixture of fish that will spawn later the same year inshore in ICNAF Div. 3L and offshore on the Southeast Shoal (ICNAF Div. 3N) (Winters 1974). It is not known whether any of these fish move inshore in the southern Avalon Peninsula (e.g. St. Mary's Bay). Thus, for a second analysis, fish collected from St. Mary's Bay were grouped with fish from Middle Cove, Conception Bay, Trinity, and Bonavista Bay while Fortune Bay was treated as a separate grouping. However, results from this second analysis did not differ from the results of the first grouping and so only results from the original analysis are presented.

Statistical analysis and results — Before investigating differences between the five stocks of capelin, the Hotelling T^2 statistic (Morrison 1976) was used to test the null hypothesis that the mean vectors of all meristic counts were identical for individuals of ages 3 and 4 of each group. Degrees of freedom (df) for pooled within group and age covariance terms was 1013 and under the null hypothesis of equal mean vectors the probabilities (P) of exceeding associated F values were <0.001 for B, <0.005 for C, >0.75 for D and E, and close to the conventional significant level of 0.05 for A. Therefore, the null hypothesis of equal mean vectors for the age groups of stocks B and C were rejected and of stocks A considered suspect.

Individual characters that contributed to the significant differences between ages were then identified on the basis of a simultaneous confidence intervals procedure (Morrison 1976). Since the differences in means of 11 individual characters alone were of interest, planned Bonferroni method (Morrison 1976) was used (as it usually yields shorter confidence intervals) with overall confidence coefficients of at least 0.95 and 0.99. The Bonferroni method provides results which are independent of those obtained from the T^2 analysis. At the 5% joint significance level the means for ventral secondary caudal rays in A and C and at the 1% joint significance level means for dorsal secondary caudal rays in A and for branchiostegals and dorsal and ventral secondary caudal rays in B differed between ages 3 and 4. No other differences between ages were significant. Groupings were then compared based on data on eight meristic characters (Table 1) designated X_j ($j = 1$ for left pectoral fin rays, 2 for dorsal fin rays, 3 for anal fin rays, 4 for pyloric caecae, 5 for upper gill rakers, 6 for lower gill rakers, 7 for precaudal vertebrae, and 8 for caudal vertebrae) with ages pooled as single samples. All eight characters were counted in each sample. Means, standard deviations, ranges of X_j, and sample sizes are given in Table 1.

Skewness of observations on each character for each

TABLE 1. Means ± standard deviations and ranges (in parentheses) of meristic characters of five groupings of capelin. Groupings of stocks correspond to those shown in Fig. 1 and Campbell and Winters (1973). Meristic characters are 1 = left pectoral fin rays, 2 = dorsal fin rays, 3 = anal fin rays, 4 = pyloric caecae, 5 = upper gill rakers, 6 = lower gill rakers, 7 = precaudal vertebrae, and 8 = caudal vertebrae.

Groupings and sample size	Meristic character	SD
Labrador–NE Nfld.		
A	1	19.14±0.75 (18–22)
156	2	13.95±0.49 (13–15)
	3	23.40±0.86 (22–26)
	4	6.17±0.89 (4–8)
	5	9.16±0.94 (8–19)
	6	27.79±1.17 (25–37)
	7	42.79±0.88 (40–46)
	8	23.72±1.03 (22–33)
N. Grand Bank–Avalon		
B	1	19.14±0.76 (17–21)
400	2	14.02±0.60 (13–18)
	3	23.46±0.85 (20–26)
	4	6.10±0.84 (3–9)
	5	9.13±0.54 (8–11)
	6	27.66±1.01 (25–31)
	7	42.81±0.99 (39–47)
	8	23.57±0.77 (20–26)
S. Grand Bank		
C	1	18.93±0.76 (18–21)
88	2	14.02±0.48 (13–15)
	3	23.20±0.80 (22–25)
	4	5.80±0.87 (4–8)
	5	9.09±0.54 (8–10)
	6	27.60±1.07 (24–30)
	7	42.81±0.73 (40–44)
	8	23.67±0.83 (22–28)
St. Pierre–Green Bank		
D	1	19.01±0.76 (17–21)
203	2	14.00±0.52 (13–15)
	3	23.52±0.81 (22–26)
	4	6.07±0.88 (4–9)
	5	9.20±0.51 (8–11)
	6	27.85±1.09 (25–30)
	7	42.92±0.89 (39–45)
	8	23.55±0.67 (22–25)
W. Nfld.		
E	1	19.32±0.74 (18–21)
234	2	13.87±0.52 (12–15)
	3	23.57±0.87 (21–26)
	4	6.12±0.82 (4–8)
	5	9.28±0.61 (8–11)
	6	27.96±1.02 (25–31)
	7	42.66±1.03 (40–45)
	8	23.69±0.80 (19–26)

stock was examined using the g_1 statistic (Snedecor and Cochran 1968). Only 13 out of 40 tests showed appreciable (g_1 values significant at $P < 0.01$) degrees of skewness. Furthermore, there was a lack of consistency in these tests in that no character showed skewness in all five stocks. It was noted that our samples and their mean values were large. Square root transformation appropriate for use when data are counts (Sokal and Rohlf 1969) did not alter these results. In addition, Seal (1964) has noted that the distribution of a linear compound of a large number of random variables would tend to be normal. Thus, skewness as a possible cause of concern in the present analysis was ruled out.

The equality of vectors of the means of eight meristic counts for the five groupings was tested by Wilk's lambda criteria (Kshirsagar 1972). The likelihood ratio statistic $U(8, 4, 1076)$ was 0.9136 ($P < 0.01$) indicating that at least two stocks differed considerably in their mean values. The mean vectors for stocks were then compared in pairs employing the T^2 statistic based on pooled within stock covariance with df = 1076. This comparison indicated that E and C are distinct from each of the other four groupings and groupings A, B, and D from a third group, designated G, such that members of G are not significantly different from one another and each member of G is distinct from C and E.

Since groupings C, E, and G differed significantly from one another, their analysis was extended to discriminant functions using the multiple regression approach with a dummy dependent variable (see e.g. Kshirsagar 1972). In analyzing data in G, the component samples A, B, and D were not pooled into a single sample; in choosing linear contrasts for discriminant function analysis, samples A, B, and D were considered as nested within G. Paired comparisons of C, E, and G were done to obtain a single linear composite of eight counts for each pair (Table 2). An analysis of variance for the discriminant functions yielded F values (df = 8 and 1069) which were significant ($P < 0.01$ for comparison C and E and for comparison E and G, and $P < 0.05$ for comparison C and G) indicating that the discriminant functions were all highly significant and provided effective separation between populations.

Discussion — Our analysis of meristic characters defined only three major stocks of capelin in the Northwest Atlantic. These can be designated as (1) Western Newfoundland (Gulf of St. Lawrence), (2) insular Newfoundland (Atlantic), and (3) Southeast Shoal. All stocks are distinct from one another and were described by discriminant functions.

The separation of the Southeast Shoal capelin from other groupings supports the conclusions of other authors (Campbell and Winters 1973; Winters 1974; Sharp et al. 1978) that this is a distinct capelin stock. This conclusion throws doubt on suggestions that capelin move to this area or inshore to spawn in different years solely in response to hydrographic conditions or that the Southeast Shoal is used as a spawning area only during times of high capelin abundance. The identification of the Southeast Shoal stock as a separate stock is significant because the interest in management

TABLE 2. Coefficients of discriminant functions, mean discriminant scores, and standard errors for capelin meristics when groupings were compared by pairs. The groupings are C = Southern Grand Bank, E = Western Newfoundland, and G = combination of A (Labrador–Northeast Newfoundland), B (Northern Grand Bank–Avalon), and D (St. Pierre–Green Bank). Discriminant coefficient subscripts correspond to the meristic characters given in Table 1.

Pair	Discriminant coefficient for meristic characters								Mean discriminant score ±SE
	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	
C G	0.1587	−0.0906	0.2188	0.2635	0.0508	0.0484	0.0112	−0.0877	8.54±0.0360 8.73±0.0123
E G	0.5199	−0.7652	0.2099	0.0099	0.3809	0.2453	−0.2399	0.1468	8.08±0.0516 7.69±0.0286
C E	0.1076	−0.1153	0.0900	0.0804	0.0591	0.0428	−0.0242	−0.0095	3.43±0.0176 3.57±0.0108

of capelin has been intense because of a dramatic decline in the catches in this fishery (Carscadden and Miller 1979). This stock is fished in ICNAF Div. 3L during its migration to the spawning grounds and on the spawning grounds on the Southeast Shoal in ICNAF Div. 3N.

Although the Southeast Shoal population is distinct, capelin spawning inshore on beaches in eastern Newfoundland could not be identified as distinct from beach spawning capelin in southern Newfoundland. Thus, we cannot evaluate the possibility that capelin spawning on the south coast of Newfoundland (where abundance was low in 1978 and 1979) mix during the spring fishery in ICNAF Div. 3L with capelin destined to spawn inshore in eastern Newfoundland or the Southeast Shoal.

Based on known seasonal migration and distribution patterns and differences in growth characteristics, Campbell and Winters (1973) and Winters (1974) suggested that there were at least four capelin stocks in the area. Thus, although our meristic analysis was more successful in identifying capelin stocks than the meristic analysis of Sharp et al. (1978), possibly because of more vigorous treatment of the data (e.g. separating the data into age-classes and identifying highly variable characters by the Bonferroni method), we would agree with the general conclusion of these authors that meristics are not the most useful means of delineating stocks of capelin. Considerable overlapping of ranges (Table 1) in all of our samples may have restricted the scope of meristics in discriminating between stocks.

Although in this analysis, meristics show only limited promise in separating capelin stocks in the Northwest Atlantic, the Bonferroni method was useful in screening and choosing characters for discriminant analysis. None of the statistical operations in this study are original in themselves but the use and sequence of these procedures offer good potential in other stock discrimination studies.

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BAILEY, R. F. J., K. W. ABLE, AND W. C. LEGGETT. 1977. Seasonal and vertical distribution and growth of juvenile and adult capelin (*Mallotus villosus*) in the St. Lawrence estuarys and Western Gulf of St. Lawrence. J. Fish. Res. Board Can. 34: 2030–2040.

CAMPBELL, J. S., AND G. H. WINTERS. 1973. Some biological characteristics of capelin, *Mallotus villosus*, in the Newfoundland area. Int. Comm. Northwest Atl. Fish. Res. Doc. 73/90, Ser. No. 3048: 3 p.

CARSCADDEN, J. E., AND D. S. MILLER. 1979. Biological aspects of capelin and a sequential capelin abundance model for the Division 3LNO stock. Int. Comm. Northwest Atl. Fish. Res. Doc. 79/11/33 (Rev.), Ser. No. 5359: 20 p.

DRAGESUND, O., AND T. MONSTAD. 1973. Observations on capelin (*Mallotus villosus*) in Newfoundland waters. Int. Comm. Northwest Atl. Fish. Res. Doc. 73/33, Ser. No. 2967: 14 p.

KOVALYOV, S. M., AND B. D. KUDRIN. 1973. Soviet investigation on capelin in the Grand Newfoundland Bank area in 1972. Int. Comm. Northwest Atl. Fish. Res. Doc. 73/26, Ser. No. 2959: 4 p.

KSHIRSAGAR, A. M. 1972. Multivariate analysis. Marcel Dekker, Inc., New York, N.Y.

MORRISON, D. F. 1976. Multivariate analysis. McGraw-Hill Book Company, New York, N.Y.

PAYNE, R. H. 1975. Esterase polymorphism in the capelin, *Mallotus villosus*: preliminary evidence for geographical variation in allele frequencies at three loci. Cons. Perm. Int. Explor. Mer C.M. 1975/H:28: 9 p.

1976. Further studies on the biochemical population genetics of the capelin, *Mallotus villosus*: demonstration that the capelin populations of West Greenland and northern America are genetically distinct. Cons. Perm. Int. Explor. Mer C.M. 1976/H:24: 7 p.

PITT, T. K. 1958. Distribution, spawning and racial studies of the capelin, *Mallotus villosus* (Müller), in the offshore Newfoundland area. J. Fish. Res. Board Can. 15: 275–293.

SEAL, H. 1964. Multivariate statistical analysis for biologists. John Wiley and Sons Inc., New York, N.Y.

SHARP, J. C., K. W. ABLE, W. C. LEGGETT, AND J. E. CARSCADDEN. 1978. Utility of meristics and morphometric characters for identification of capelin (*Mallotus villosus*) stocks in Canadian Atlantic waters. J. Fish. Res. Board Can. 35: 124–130.

SNEDECOR, G. W., AND W. G. COCHRAN. 1968. Statistical methods. Iowa State Univ. Press, Ames, Iowa.
SOKAL, R. R., AND F. J. ROHLF. 1969. Biometry. W. H. Freemand and Company, San Francisco, Calif.
TAYLOR, W. R. 1967. An enzyme method of cleaning and staining small vertebrates. Proc. U.S. Nat. Mus. 122: 17 p.
TEMPLEMAN, W. R. 1948. The life history of the capelin (*Mallotus villosus*) Müller) in Newfoundland waters. Nfld. Govt. Lab. Bull. 17: 151 p.
WINTERS, G. H. 1974. Rationale for partition of capelin quota in Subareas 2 and 3. Int. Comm. Northwest Atl. Fish. Res. Doc. 74/12, Ser. No. 3158: 2 p.
WINTERS, G. H., AND J. E. CARSCADDEN. 1978. Review of capelin ecology and estimation of surplus yield from predator dynamics. Int. Comm. Northwest Atl. Fish. Res. Bull. 13: 21–30.

Modified Dimethyl Sulfoxide (DMSO) Extraction for Chlorophyll Analysis of Phytoplankton

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BURNISON, B. K. 1980. Modified dimethyl sulfoxide (DMSO) extraction for chlorophyll analysis of phytoplankton. Can. J. Fish. Aquat. Sci. 37: 729–733.

This new extraction procedure eliminates the need for grinding glass fiber filters by heating the filter in DMSO (4 mL) for 10 min at 65°C and subsequently filtering or centrifuging the sample after dilution with 90% acetone (6 mL). *Scenedesmus* and *Selenastrum* species gave about 20% higher concentrations of chlorophyll *a* using this method as compared to the standard 90% acetone procedure or grinding in DMSO only. The difference between heated DMSO and 90% acetone was reduced when natural phytoplankton were analyzed.

Key words: chlorophyll, Chlorophyta, dimethyl sulfoxide, acetone, extraction

BURNISON, B. K. 1980. Modified dimethyl sulfoxide (DMSO) extraction for chlorophyll analysis of phytoplankton. Can. J. Fish. Aquat. Sci. 37: 729–733.

Avec cette nouvelle méthode d'extraction, on peut se dispenser du broyage de filtres en fibre de verre en chauffant le filtre dans du DMSO (4 mL) durant 10 min à 65°C et ensuite en filtrant ou en centrifugeant l'échantillon après dilution avec acétone à 90% (6 mL). En utilisant cette méthode plutôt que la méthode standard avec acétone à 90% ou broyage dans le DMSO seulement, les espèces de *Scenedesmus* et de *Selenastrum* donnent des concentrations de chlorophylle *a* d'environ 20% supérieures. Dans des analyses de phytoplancton naturel, la différence entre la méthode au DMSO chauffé et à l'acétone à 90% est réduite.

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CHLOROPHYLLOUS pigments from phytoplankton are extracted by various organic solvents. Extraction efficiency of 90% acetone is inferior to dimethyl sulfoxide : acetone (Shoaf and Lium 1976; Stauffer et al. 1979) and methanol (Talling and Driver 1963; Marker 1972; Holm-Hanson and Riemann 1978). The specific absorption coefficient (ϵ) for chlorophyll *a* in methanol is still contradictory, since the values given by Talling and Driver (1963), Riemann (1978), and Stauffer et al. (1979) are 74.9, 77.4, 83.9 $L \cdot g^{-1} \cdot cm^{-1}$, respectively. These values are based on the extinction coefficient of chlorophyll *a* in 90% acetone at 664 nm of 87.67 $L \cdot g^{-1} \cdot cm^{-1}$ (Jeffrey and Humphrey 1975). The absorbance spectra for pheophytin *a* in methanol is very pH dependent (Livingstone et al. 1953; Marker 1972) and acidified samples must be neutralized before the absorbance is read. The wavelength shift for pheophytin *a* is even more pronounced when measured fluorometrically, but neutralization of the acidified methanol extracts was not recommended (Holm-Hansen and Riemann 1978).

Dimethyl sulfoxide (DMSO) was superior to 90% acetone for the extraction of chlorophyll *a* and *b* from certain freshwater green algae, but diatoms and blue-greens were extracted with equal efficiency by both solvents (Shoaf and Lium 1976). Stauffer et al. (1979), however, showed DMSO:acetone superior to 90% acetone in extracting certain blue-green algae. Shoaf and Lium (1976) showed that the absorbance of chlorophyll *a* and *b* were the same between 600 and 750 nm in 90% acetone as in a 1:1 (v/v) mixture of

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DMSO and 90% acetone. The ϵ which measured accurately in 90% acetone can therefore be used for the DMSO/acetone mixture. The acid factor (E_{664}/AE_{664}) was claimed to be the same for these two solvents. The basic fault of their recommended procedure, however, is the type of filter used to concentrate the algae. In the present paper, filter types are compared and a modified DMSO extraction procedure is outlined which eliminates the grinding step. Grinding not only is tedious but it may lead to a loss of samples when the pestle "jams" in the mortar. The proposed method is tested with two cultured green algae, *Selenastrum capricornutum* and *Scenedesmus basiliensis*, and natural phytoplankton populations from "limnocorral" nutrient experiments at Lake St. George, Ont.

Materials and methods — Description of method — Natural or cultured phytoplankton are filtered onto glass fiber filters (Whatman GF/C or GF/F, 42.5 mm diameter). The filters are loosely rolled, placed in vials (15 mm × 45 mm) with Teflon-lined screw caps, and may be stored at −20°C at this point for later analysis. For analysis, the vials are brought to room temperature, 4 mL of DMSO (reagent grade) are added, and the vials are resealed. They are placed in a heating block (Temp-Block module heater, Lab-Line Instruments, Melrose Park, Ill.) at 65°C for 10 min. The vials are then removed, agitated thoroughly, and the contents filtered either through a Nuclepore 0.2-μm membrane filter or Whatman GF/C filter. The vial and filter are rinsed with 90% acetone and the filtrate volume brought to 10 mL with 90% acetone. Spectrophotometric readings are taken in a 5-cm cuvette with a Beckman model 25 spectrophotometer at 750 and 664 nm (647, 630, and 480 nm, optional readings for trichromatic formula). The extract is then acidified with 10 μL concentrated HCl and the absorbances reread at 750 and 664 nm after 4 or 5 min. This acid addition results in a final concentration of 0.012 N HCl when it is added to the 10-mL extract as recommended by Marker (1972).

The equation of Lorenzen (1967) is used to calculate the concentration of pheopigment-corrected chlorophyll *a* and pheopigments (pheophytin and pheophorbide) using the Jeffrey and Humphrey (1975) extinction coefficient for chlorophyll *a*.

The possibility of degradation of chlorophylls in heated DMSO was tested using purified chlorophylls *a* and *b* (Sigma Chemical Co., St. Louis, Mo.). The chlorophylls were dissolved in diethyl ether. Fifty microlitres of each solution was added to either 10 mL of 90% acetone or to 4 mL DMSO. The DMSO solution was heated at 65°C for 10 min in a closed vial and then brought to a final volume of 10 mL with 90% acetone. Absorbances were taken at various wavelengths before and after acidification. Chlorophyll *a* solutions were acidified with 10 μL concentrated HCl. The chlorophyll *b* solutions were acidified with 20 μL concentrated HCl, because the addition of 10 μL was insufficient to remove completely the Mg^{2+} from the chlorophyll *b* molecule within the allotted 4 to 5 min.

Cultured algae experiments — The effect of temperature and time on the extraction efficiency of DMSO on *S. basiliensis* was determined by extracting the algae concentrated on glass fiber filters (Whatman GF/C) in duplicate at various temperatures (extraction time was 10 min) and times (extraction temperature was 65°C). *Scenedesmus basiliensis* was also used as a source of chl *a* to compare the proposed technique to the DMSO methods of Shoaf and Lium (1976) and Stauffer et al. (1979); filtration was used for clarification.

The compatibility of cellulose membrane filters with the proposed DMSO method and the 90% acetone method (Kerr and Subba Rao 1966) was tested using Millipore HA (mixed cellulose esters), Sartorius SM 11106 (cellulose acetate), and SM 11306 (cellulose nitrate). Pigments were extracted from *Selenastrum capricornutum* on the various filters without the use of $MgCO_3$. These results were compared to extracts of the alga concentrated on Whatman GF/F filters.

Natural phytoplankton sampling — Four replicate samples were obtained from 12 different stations in Lake St. George, Ont., during the summer of 1977. Ten of these stations were 'limnocorral" experimental sites where nutrient loadings of phosphorus, nitrate, and ammonia were being investigated. The other two were mid-lake stations. Duplicate samples were filtered onto GF/F filters and extracted with 90% acetone or DMSO as described above.

Results and discussion — The optimum temperature for heat extraction of chlorophyll *a* from *Scenedesmus basiliensis* with DMSO was 65°C. This was the minimum temperature for the best extraction, and heating to 80°C did not seem to affect the absorbancy of the extracts adversely. At 65°C, the optimum extraction time was 10 min, but the maximum time tested (15 min) was equally effective. The vials must be at room temperature before they are placed in the heating block because vials at −20°C would take longer to reach the optimum 65°C.

The proposed DMSO method for chlorophyll *a* extraction is more efficient and reproducible than grinding in DMSO (Shoaf and Lium 1976) or 90% acetone (Table 1). The method of Stauffer et al. (1979) gives slightly lower absorbance values ($P = 0.001$), but the difference is only 5%. However, their procedure requires 40 mL of extracting solvent (necessitating larger sample volumes or decrease in sensitivity), an extraction time of 40 h, and a precise decantation technique.

Extraction of algae with DMSO does not degrade the chlorophyllous pigments (Shoaf and Lium 1977).

TABLE 1. Comparison of DMSO extraction procedures with 90% acetone extraction using glass fiber filters. Mean (n = 5); SD, standard deviation.

Method	Extraction time	Absorbance (663 nm) $\bar{x} \pm$ SD
Proposed DMSO	10 min	0.430 ± 0.006
Ground in DMSO[a]	> 10 min	0.359 ± 0.018
Acetone: DMSO[b]	40 h	0.407 ± 0.005
Ground in 90% acetone	2 h	0.339 ± 0.017

[a]After Shoaf and Lium (1976); ground in DMSO and allowed to stand for at least 10 min before filtration.

[b]After Stauffer et al. (1979); 40 mL acetone: DMSO (1:1) solvent added to filter and allowed to stand ~40 h before filtration.

TABLE 2. Purified chlorophylls *a* and *b* absorbances at various wavelengths in heated (see Materials and methods) DMSO/90% acetone (solvent 1) and 90% acetone (solvent 2). Data are averages of four replicates. SD, standard deviation; CV, coefficient of variation.

	Mean absorbance		SD		CV (%)	
	Solvent 1	Solvent 2	Solvent 1	Solvent 2	Solvent 1	Solvent 2
Chlorophyll *a*						
Wavelength						
664 (λ_{max})	0.251	0.253	0.002	0.002	0.76	0.88
647	0.069	0.065	0.003	0.002	3.86	3.32
630	0.045	0.043	0.003	0.002	7.48	4.65
480	0.016	0.010	0.002	0.003	15.31	32.72
431 (λ_{max})	0.314	0.298	0.009	0.001	2.88	0.42
Acidification						
*A*664	0.145	0.142	0.001	0.001	0.98	0.89
Acid factor$_{664}$[a]	1.728	1.783	0.024	0.006	1.39	0.34
Chlorophyll *b*						
Wavelength						
664	0.055	0.045	0.001	0.002	1.75	4.06
647 (λ_{max})	0.203	0.205	0.004	0.003	1.82	1.52
630	0.060	0.059	0.001	0.003	2.17	4.99
480	0.159	0.124	0.002	0.003	1.31	2.23
459 (λ_{max})	0.588	0.591	0.007	0.008	1.18	1.27
Acidification						
*A*664	0.071	0.063	0.002	0.002	2.72	2.39
*A*654 (λ_{max})	0.140	0.136	0.003	0.003	2.21	1.84

[a] E_{664}/AE_{664}.

These pigments can potentially be degraded by four processes: epimerization, allomerization, phytolization, and acidification. When the parent chlorophylls (*a* and *b*) are heated, an equilibrium mixture of *a* and *a'*, and *b* and *b'* will result (Strain and Manning 1942). The epimers (*a'* and *b'*) have an almost identical spectrum (Strain 1954) and since there is not a change in the chemical composition or molecular weight for these epimers, the specific absorbance coefficients would not be expected to change in heated DMSO. Table 2 shows there are no significant changes in the absorbance spectra for chlorophylls *a* and *b* in heated DMSO (solvent 1) as compared to 90% acetone (solvent 2) at the selected wavelengths (664, 647, 630 nm). Chlorophyll *b* exhibited a 28.2% increase in absorbance at 480 nm in DMSO/90% acetone over that in 90% acetone. The effect on the determination of carotenoids was not examined.

Allomerized (oxidized) chlorophylls have a red absorbance maximum about 10 nm shorter than the parent chlorophylls and even trace amounts of water inhibit the allomerization of chlorophylls (Strain 1954). A shift in the λ_{max} was never observed in solvent 1 (Table 2) and there is always water present in the samples in addition to the 0.2% water in the DMSO. Therefore, chlorophylls in solvent 1 should not be readily oxidized, except perhaps by photooxidation.

The enzyme chlorophyllase is responsible for phytolization of chlorophylls yielding chlorophyllides. The activity of this enzyme in DMSO at 65°C is not known, but its activity in acetone and methanol extracts of marine phytoplankton could not be measured (Moreth and Yentsch 1970). The acid factor (E_{664}/AE_{664}) decreased by 3.1% in DMSO/90% acetone when compared to 90% acetone (Table 2) and is considered insignificant. When HCl is added to remove the Mg^{2+} from chlorophyll *a*, there is the characteristic shift in the red absorbance maximum from 663 to 666 nm (Vernon 1960; Marker 1972) in both solvents.

Choice of filter and solvent — The most difficult phytoplankton from which to extract chlorophyllous pigments are the Chlorophyceae (green algae). Both the type of filter used to concentrate the algae (glass fiber or cellulose ester membrane) and the solvent used affect the efficiency. Long and Cooke (1972) noted unextracted pigments from algal samples retained on membrane filters when using 90% acetone. Therefore, the differences between solvents can only be compared when the type of filter behaves similarly in the solvents tested. Since glass fiber filters are insoluble in all organic solvents, this condition is met. The extraction efficiencies of heated DMSO/90% acetone (4:6) and 90% acetone are compared in Table 3. The differences between the two solvents are 23% for *Scenedesmus basiliensis* and 17% for *Selenastrum capricornutum* when glass fiber filters are used. Shoaf and Lium (1976) reported a 2.3-fold difference between DMSO/90% acetone (1:1) and 90% acetone for *Scenedesmus quadricauda*, and a 59-fold difference for *Selenastrum*

TABLE 3. Comparison of membrane and glass fiber filters. Mean (n = 5); SD, standard deviation; CV, coefficient of variation. Solvent 1 = DMSO/90% acetone (4:6); solvent 2 = 90% acetone.

Alga	Filter	Solvent	Chlorophyll a (μg/L) $\bar{x} \pm$ SD	CV (%)
Selenastrum capricornutum	Whatman GF/F	1	2003 ± 106	5.3
		2	1710 ± 287	16.8
	Millipore HA	1	2061 ± 101	4.9
		2	894 ± 171	19.1
Scenedesmus basiliensis	Whatman GF/F	1	1064 ± 36	3.4
		2	866 ± 56	6.5

capricornutum. The results are undoubtedly erroneous because they used cellulose ester membrane filters (brand name was not mentioned).

DMSO is a superior solvent for membrane filters, completely dissolving all cellulose ester membrane filters tested. The 90% acetone was unable to dissolve cellulose acetate (Sartorius SM 11106) and only appeared to dissolve the cellulose nitrate (Sartorius SM 11306) and the mixed cellulose acetate and nitrate ester filters (Millipore HA) within 10 min. Microscopic observations of the cells showed that they were surrounded by immiscible fluid (partially solubilized cellulose ester filter). The barrier prevents the acetone from penetrating the cell and subsequently yielding low chlorophyll values. When the samples were allowed to extract in 90% acetone for longer times (16 h), the chlorophyll concentrations increased significantly but they never attained the values obtained by DMSO extraction.

The standard deviations and coefficients of variation (CV) of the proposed DMSO and 90% acetone methods for pheophytin corrected chl *a* are compared in Table 3. The CV using Whatman GF/F filters is 5.3 and 16.8% for the proposed method and 90% acetone method, respectively, using *S. capricornutum* and 3.4 and 6.5% for *Scenedesmus basiliensis*, showing the proposed method to be more reproducible.

The DMSO extraction can also be done in centrifuge tubes (water bath at 65°C), 90% acetone added to 10 mL after 10 min., and tubes centrifuged at 5000 *g* for 10 min. The only transfer step would be to the cuvette.

Natural phytoplankton — The difference between ground 90% acetone and heated DMSO extracts of chlorophyll *a* from natural phytoplankton (Lake St. George, 1977) was very small. Table 4 illustrates how similar the two methods of extraction were for most sampling stations. The consistent exception were samples from the shallow limnocorrals. These limnocorrals were dominated by the green alga *Lagerheimia* for most of the summer. The superior extraction of chl *a* by heated DMSO confirm the laboratory results from other green algae.

TABLE 4. Phytoplankton chlorophyll *a* in Lake St. George and limnocorrals (May to October 1977).

	Depth	n	Linear regression equation[c]	r	Probability of a slope = 1.00
A[a]	Epilimnion	54	$Y = 1.02X - 0.608$	0.972	>0.50
A	Metalimnion	43	$Y = 0.98X + 0.003$	0.984	0.50
A	Hypolimnion	45	$Y = 1.07X - 0.056$	0.965	0.14[d]
B[b]	Integrated	48	$Y = 0.89X + 0.673$	0.986	<0.001
Total	—	190	$Y = 0.91X + 0.741$	0.983	<0.001

[a]A = deep corrals and Station A (average depth 14 m).
[b]B = shallow corrals and Station B (average depth 4 m).
[c]Chlorophyll *a* concentrations (μg/L) extracted by X (heated DMSO) and Y (ground in 90% acetone).
[d]At this location the 90% acetone extractions were higher.

The extrapolation of algal culture differences to field samples is often difficult. Yentsch (1970) has shown a significant difference between natural phytoplankton populations and cultured marine phytoplankton. He used chromatographic and petroleum ether/methanol solvent partition to illustrate the presence of the nonpolar decomposition products of chlorophyll (chlorophyllide and pheophorbide). The spectrophometric technique proposed by Lorenzen (1967) and used in the present study does not distinguish between chlorophyll *a* and chlorophyllide *a* and may overestimate chlorophyll *a* occasionally. Hallegraeff (1976) reported that in three lakes in the Netherlands the Mg-containing derivatives of chlorophyll *a* (isomeric and allomeric chlorophyll *a*, in addition to chlorophyllide *a*) were more abundant than the pheopigments, and he recommended chromatographic analyses of the cell pigments as the only means of estimating photosynthetically active chlorophyll *a*. However, chromatographic techniques are laborious and the solvent partition technique of Yentsch (1970) or that of Whitney and Darley (1979) seem to be adequate to separate the nonpolar chlorophyll derivatives (lacking the phytol group) from the parent chlorophylls. Using DMSO instead of acetone as the extracting solvent does not interfere with the solvent partitioning.

In conclusion, the maximum difference between chlorophyll *a* concentrations extracted by heated DMSO and 90% acetone is 25% based on the present observation of two cultured green algae. A 35% difference between A-DMSO and 90% acetone extractions of a blue-green algal population was noted by Stauffer et al. (1979) but they did not grind in 90% acetone. The goal of chlorophyll extractions is complete removal of chlorophyllous pigments, and heating in DMSO provides a quick, efficient, and reproducible extraction solvent system.

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HALLEGRAEFF, G. M. 1976. Pigment diversity in freshwater phytoplankton. I. A comparison of spectrophotometric

and paper chromatographic methods. Int. Rev. Gesamten Hydrobiol. 61: 149–168.

HOLM-HANSEN, O., AND B. RIEMANN. 1978. Chlorophyll *a* determination: improvements in methodology. Oikos 30: 438–447.

JEFFREY, S. W., AND G. F. HUMPHREY. 1975. New spectrophotometric equations for determining chlorophylls *a*, *b*, c_1 and c_2 in higher plants, algae and natural phytoplankton. Biochem. Physiol. Pflanz. 167: 191–194.

KERR, J. D., AND D. V. SUBBA RAO. 1966. Extraction of chlorophyll *a* from *Nitzschia closterium* by grinding. Monogr. Oceanogr. Methodol. (UNESCO, Paris), 1: 65–69 p.

LIVINGSTONE, R., R. PARISER, L. THOMPSON, AND A. WEBBER. 1953. Absorption spectra of solutions of pheophytin *a* in methanol containing acid or base. J. Am. Chem. Soc. 75: 3025–3026.

LONG, E. B., AND G. D. COOKE. 1972. A quantitative comparison of pigment extraction by membrane and glass-fiber filters. Limnol. Oceanogr. 16: 990–992.

LORENZEN, C. J. 1967. Determination of chlorophyll and pheo-pigments: spectrophotometric equations. Limnol. Oceanogr. 12: 343–346.

MARKER, A. F. H. 1972. The use of acetone and methanol in the estimation of chlorophyll in the presence of phaeophytin. Freshwater Biol. 2: 361–385.

MORETH, C. M., AND C. S. YENTSCH. 1970. The role of chlorophyllase and light in the decomposition of chlorophyll from marine phytoplankton. J. Exp. Biol. Ecol. 4: 238–249.

RIEMANN, B. 1978. Absorption coefficient for chlorophylls *a* and *b* in methanol and a comment on interference of chlorophyll *b* in determinations of chlorophyll *a*. Vatten 3: 187–194.

SHOAF, W. T., AND B. W. LIUM. 1976. Improved extraction of chlorophyll *a* and *b* from algae using dimethyl sulfoxide. Limnol. Oceanogr. 21: 926–928.

1977. The quantitative determination of chlorophylls *a* and *b* from freshwater algae without interference from degradation products. J. Res. U.S. Geol. Surv. 5: 263–264.

STAUFFER, R. E., G. F. LEE, AND D. E. ARMSTRONG. 1979. Estimating chlorophyll extraction biases. J. Fish. Res. Board Can. 36: 152–157.

STRAIN, H. H. 1954. Oxidation and isomerization reactions of the chlorophylls in killed leaves. Agric. Food Chem. 2: 1222–1226.

STRAIN, H. H., AND W. M. MANNING. 1942. Isomerization of chlorophylls *a* and *b*. J. Biol. Chem. 146: 275–276.

TALLING, J. F., AND D. DRIVER. 1963. Some problems in the estimation of chlorophyll *a* in phytoplankton, p. 142–146. *In* M. S. Doty [ed.] Conference of primary productivity measurement, marine and freshwater, Hawaii, 1961. U.S. Atomic Energy Comm. TID-7633.

VERNON, L. P. 1960. Spectrophotometric determination of chlorophylls and pheophytins in plant extracts. Anal. Chem. 32: 1144–1150.

WHITNEY, D. E., AND W. M. DARLEY. 1979. A method for the determination of chlorophyll *a* in samples containing degradation products. Limnol. Oceanogr. 24: 183–186.

YENTSCH, C. S. 1970. The state of chlorophyll in the aquatic environment. Proc., Prediction and measurement of photosynthetic productivity, Trebon, 1969. p. 489–502.

Statistical Problems in Studies of Temperature Preference of Fishes

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When the laboratory determined acute temperature preference vs. acclimation relationship of fishes is analyzed, the correct assessment of degrees of freedom is central to analyses of covariance, analysis of variance, and regression analysis. Many investigators have assumed that recordings of fish positions in a horizontal temperature gradient trough made at 1-min intervals in a test of 20-min duration represent 20 degrees of freedom. Such an assumption can arbitrarily allow an investigator to increase or decrease the number of degrees of freedom by simply shortening or lengthening the time intervals for observation of the position of fish in the trough. We contend that each temperature preference test provides only one data point (single degree of freedom) even though an investigator may have used more than one fish in a test. Failure to recognize this distinction may lead to erroneous conclusions from data. For illustrative purposes, we reanalyzed three sets of published data using the correct number of degrees of freedom. The reanalysis showed that the significant values were overstated.

Key words: statistics, analysis, sample size, temperature, preference, fishes

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Dans l'analyse de la relation entre température préférée et acclimatation chez les poissons, déterminée en laboratoire, la détermination exacte des degrés de liberté est essentielle aux analyses de covariance, de variance et de régression. Plusieurs chercheurs ont supposé que le fait de noter la position des poissons dans un bassin à gradient horizontal de températures à intervalles de 1 min dans un essai d'une durée de 20 min représentent 20 degrés de liberté. Une telle hypothèse peut arbitrairement permettre à un chercheur d'augmenter ou de diminuer le nombre de degrés de liberté simplement en raccourcissant ou en prolongeant l'intervalle de temps entre les observations de position des poissons dans le bassin. Nous soutenons que chaque essai de préférence de température ne donne qu'un point (un seul degré de liberté), même si l'observateur utilise plus d'un poisson par essai. Si l'on ne fait pas cette distinction, on risquera des conclusions erronnées à partir des données. Comme exemple, nous réanalysons trois séries de données publiées en utilisant le nombre exact de degrés de liberté. Cette nouvelle analyse montre que la signification statistique avait été surestimée.

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CONCERNS over the effects of heated discharges into natural waters have stimulated numerous studies of laboratory determined acute temperature preferences of fishes (Cherry et al. 1975, 1977; Coutant 1977; Hall et al. 1978; Stauffer et al. 1975). Some general conclusions of these studies and reviews are that the temperature preferences of fishes vary with acclimation temperature, between rising versus falling field temperatures, and geographic areas. Such conclusions were based upon the analysis of data primarily via analysis of covariance (ANCOV), analysis of variance (ANOVA), and regression analysis. Central to the interpretation of the results of such analyses is the concept of degrees of freedom. In general, the total number of degrees of freedom in ANCOV, ANOVA, or regression analysis is the number of independent data points. Incorrect assessment of degrees of freedom can lead to erroneous interpretations. Acute temperature preference studies seem to be particularly prone to such misinterpretations. We have selected three recent publications (Cherry et al. 1975, 1977; Hall et al. 1978) to illustrate these statistical problems and offer a reanalysis of the same data with what we view as the correct number of degrees of freedom. Undoubtedly, many similar analyses exist in unpublished reports, nonrefereed papers, proceedings, etc. Hall et al. (1978) provided the most complete data set for a reanalysis. Because of some limitations imposed by the data of Cherry et al. (1975), 1977) reanalysis of these data is necessarily more restricted.

Previous methods and analysis — Hall et al. (1978) determined the acute temperature preferences of three geographic populations of white perch, *Morone americana*, at various acclimation temperatures. The objective of the study was to determine if significant differences occur in temperature preferences of the three populations. They obtained responses of 128 fish, tested one at a time. The position of each fish was recorded each minute for 20 min. They treated these 20 records as 20 independent measurements. However, they considered the mean of each 20 recordings on each fish as an indicator of preferred temperature. These data are reproduced here in Table 1.

Hall et al. (1978) analyzed the data as though there were 2560 independent observations or 2559 df (Table 2). From the analysis of covariance the investigators con-

TABLE 1. Temperature (°C) preference data for three populations of *Morone americana* used for analysis. Preference temperature is average of 20 recordings on each fish. Data reproduced from Hall et al. (1978).

	North Carolina acclimation temp (°C)						Maryland acclimation temp (°C)						New Jersey acclimation temp (°C)					
Fish	6	12	18	24	30	33	6	12	18	24	30	33	6	12	18	24	30	33
1	9.9	20.9	24.8	32.1	28.9	31.2	17.0	15.8	29.2	26.9	30.1	31.7	17.2	17.5	21.3	29.2	30.8	29.5
2	18.6	21.2	25.2	29.0	30.6	31.7	16.5	14.0	18.5	25.3	34.8	32.5	16.3	18.6	26.0	29.2	29.0	29.2
3	17.0	18.1	26.6	28.3	28.3	33.3	17.7	23.5	19.9	27.3	30.5	30.5	17.0	15.9	23.4	29.6	30.8	29.8
4	20.3	20.8	23.6	30.4	29.5	33.7	16.0	29.0	21.2	26.3	30.5	30.2	19.0	17.4	25.1	26.1	30.5	30.3
5	18.5	19.5	24.2	27.5	30.5	33.0	11.2	25.1	23.6	27.2	30.2	31.3	21.0	19.6	21.1	32.5	26.5	31.6
6	15.7	23.3	24.4	29.8	30.0	32.8	12.6	21.0	19.7	25.2	29.6	29.7	12.0	22.8	29.2	32.5	28.4	29.2
7	16.7	21.8	24.9	30.8	28.3	—	—	—	—	—	—	—	15.9	17.7	28.7	27.0	—	30.4
8	17.8	20.4	24.0	31.8	31.1	—	—	—	—	—	—	—	17.0	23.0	17.5	28.7	—	30.1
x =	16.8	20.7	24.7	30.0	29.6	32.6	15.2	21.4	22.0	26.3	31.0	30.7	16.9	19.0	24.0	29.3	29.3	30.0

TABLE 2. Comparison of results of the analysis of covariance: Hall et al. (1978) vs. new analysis. Data in Table 1 used for new analysis.

Source of variation	df	Sum of squares	Mean squares	F[a]	Probability of significance
		Hall et al. (1978)			
Acclimation	1	71974.195	71974.195	70.845**	0.001
Location	2	990.040	495.020	42.441**	0.001
Error	2556	29812.134	11.664		
Total	2559	102776.369			
		New analysis			
Acclimation	1	3586.533	3586.533	502.24**	0.001
Location	2	36.995	18.498	2.59 NS	0.079
Error	124	885.467	7.141		
Total	127	4508.995			

[a]**Significant at $P < 0.01$. NS, nonsignificant ($P > 0.05$).

cluded that significant differences ($P < 0.0001$) in temperature preferences occur among the three populations.

Cherry et al. (1975, 1977) studied the acute temperature preferences of 13–15 species collected from the New and East Rivers, Virginia, during rising and falling field temperatures. They used eight specimens of a species in each test and recorded the temperature occupied by fishes in the horizontal temperature gradient trough at 2-min intervals for 40 min. They replicated each test once at each acclimation temperature. Table 3 shows the average preferred temperatures for selected species at various acclimation temperatures.

They reported that results of the statistical analyses revealed significant differences ($P < 0.05$) in temperature preferences of the three species (stoneroller, *Campostoma anomalum*, fathead minnow, *Pimephales promelas*, bluegill, *Lepomis macrochirus*) between the rising and falling field temperatures. Other species did not show significant differences ($P > 0.05$).

New analysis — As in the case of analysis by Hall et al. (1978), acclimation temperature may be considered as a continuous variable and thus has one degree of freedom associated with it. The data used for this model were taken from Table 1 and contained a total of 128 independent measurements. The analysis of covariance using the General Linear Models procedure in the Statistical Analysis System (Barr et al. 1976) was used. The analysis (Table 2) revealed that the effect of location was not significant ($P > 0.05$). The sum of squares due to location contributed less than 1% of the total sum of squares. Consequently, differences attributed by Hall et al. (1978) to the geographical origin of white perch populations may have in fact been of a random nature. For comparison we have also reproduced the analysis of Hall et al. (1978) in Table 2. Note that in their analysis also the sum of squares due to location comprised less than 1% of the total sum of squares; location has little predictive value.

The use of 2559 df by Hall et al. (1978) inflated the sum of squares due to location over 20 times (990.040 vs. 36.995) and the Mean Square (MS) was similarly falsely inflated. If this artificially inflated MS were tested against the error term, the resulting F-ratio would be significant ($P < 0.001$).

Because of the incorrect assessment of the number of degrees of freedom by Hall et al. (1978), we have revised the reported regression equations for preference vs. acclimation for each population (Table 4). The values of R^2 (variation explained) in the two sets of equations differ by as much as 9%.

We used the above model to reanalyze the data of

TABLE 3. Average preferred temperature of three fish species during the rising and falling field temperature conditions. Data reproduced from Cherry et al. (1975, 1977).

		Acclimation temperature (°C)										
		6	9	12	15	18	21	24	27	30	33	36
Fathead minnow	Rising	—	—	19.5	21.2	20.9	22.0	25.4	27.6	28.7	—[b]	—[b]
	Falling	—[c]	—[c]	19.8	21.3	22.1	23.8	26.6	28.9	—[b]	—	—
Stoneroller	Rising	—	—	16.5	17.0	21.0	22.4	25.1	28.2	27.4	—[b]	—[b]
	Falling	13.4	15.2	20.7	21.5	22.3	23.6	25.3	28.6	—[b]	—	—
Bluegill	Rising	—	—	24.1	25.2	26.8	27.8	28.2	30.0	32.4	30.9	31.8
	Falling	18.7	19.6	23.9	25.9	29.2	30.1	31.2	31.4	31.7	—	—

[a]Fish obtained from state hatcheries.
[b]Acclimation temperature approached or exceeded lethal limits.
[c]No fish available.

TABLE 4. Comparison of regression equations (preference vs. acclimation) for the three populations of white perch: Hall et al. (1978) vs. new analysis. Data in Table 1 used for new analysis. P = preference, A = acclimation, R^2 = variance explained; $S_{y.x}$ = standard error of estimate.

State	Linear	R^2	$S_{y.x}$	Quadratic	R^2	$S_{y.x}$
			Hall et al. (1978)			
New Jersey (n = 920)	P = 13.53+0.544A	0.83	N/A[a]	10.31+0.97A−0.011A²	0.84	N/A
Maryland (n = 721)	P = 12.36+0.572A	0.85	N/A	12.42+0.57A+0.0002A²	0.85	N/A
North Carolina (n = 919)	P = 14.04+0.566A	0.85	N/A	11.35+0.925A−0.009A²	0.85	N/A
			New analysis			
New Jersey (n = 46)	P = 13.94+0.530A	0.77	2.830	10.56+0.978A−0.011A²	0.79	2.730
Maryland (n = 36)	P = 12.62+0.579A	0.76	3.173	11.70+0.701A−0.003A²	0.76	3.213
North Carolina (n = 46)	P = 14.03+0.572A	0.88	2.052	11.09+0.967A−0.010A²	0.89	1.937

[a]N/A, not available.

Cherry et al. (1975, 1977) (Table 3) for the three species where they reported significant differences ($P < 0.05$). Evidently, Cherry et al. (1975, 1977) also inflated the degrees of freedom because our reanalysis does not support the significant levels as reported (Table 5). Note that only in the case of stoneroller did the effect of rising and falling field temperatures (field) approach significance ($P = 0.01$). Indeed, the effect of field nearly vanished for the other two species. In our analysis the sum of squares due to direction of field temperatures contributed less than 2% of the total variation. Consequently, for predictive purposes the variation explained by field is negligible.

Discussion — This note has attempted to address the statistical problems associated with repeated measurements on the same specimen(s) with regard to the appropriate degrees of freedom in acute temperature preference studies. The suggested statistical method provides an avenue to arrive at correct conclusions from a data set. Overestimation of degrees of freedom in the examples cited herein caused significant values to be overstated by authors.

There is no reason to believe that the response of one fish affected the behavior of any other fish because only one fish was tested at a time by Hall et al. (1978). However, when the position of the same fish is recorded repeatedly there will exist a correlation among the observations on that fish. Thus, the 20 measures per fish are not independent. There is a difference between testing 128 fish 20 times each and testing 2560 fish once each, but the analysis as reported by Hall et al. (1978) failed to recognize this difference. Similarly, Cherry et al. (1975, 1977) seem to have misinterpreted the

TABLE 5. Analysis of covariance for determining difference in temperature preferences of three fish species during the rising and falling field temperature conditions. Data from Cherry et al. (1975, 1977) shown in Table 4 used for analysis.

Source of variation	df	Sum of squares	Mean squares	F	Probability of significance
			Fathead minnow		
Acclimation	1	125.49	125.49	153.21	0.0001
Field[a]	1	3.02	3.02	3.69	0.084
Error	10	8.19	0.81		
Total	12	234.34			
			Stoneroller		
Acclimation	1	283.96	283.40	192.34	0.0001
Field[a]	1	11.56	71.56	7.84	0.016
Error	12	17.68	1.47		
Total	14	312.64			
			Bluegill		
Acclimation	1	242.67	242.67	87.09	0.0001
Field[a]	1	4.48	4.48	1.61	0.224
Error	15	41.79	2.79		
Total	17	288.95			

[a]Rising vs. falling field temperatures.

correct number of independent measures in their studies.

The use of 2559 total df by Hall et al. (1978) is incorrect for other reasons as well. From the biological viewpoint it violates the notion of the meaning of preference. By definition, fishes do not prefer a given temperature for 1 or 2 min only; preference is a function of frequency with which each temperature is occupied in a temperature gradient trough. The most frequently occupied temperature is a proper indicator of the preferred temperature in a test. Thus, some measure of central tendency (mean, mode, or median) of the frequency distribution of temperatures occupied should be employed as a measure of preference. If this were not the case an investigator could arbitrarily increase or decrease his sample size (degrees of freedom) simply by shortening or lengthening the time intervals of recording the position of the fish(s).

Even if Hall et al. (1978) had conducted 2560 independent tests on the white perch, the results may not have been biologically significant or of much predictive value. The sensitivity to detect small differences (less than 1.5°C in Hall et al.) would be substantially increased. The contribution of the sum of squares due to location to the total variation was less than 1% in analysis presented by Hall et al. (1978); highly significant statistically ($P < 0.0001$) although hardly so biologically. Consequently, from a practical standpoint we believe that detection of differences of such small magnitude considering the time, effort, and expenditures involved in such studies would not be a worthwhile undertaking. Often in temperature studies differences of 1 or 2°C are not regarded as significant. Note the variation in preferred temperature within each acclimation (Table 1).

Another problem to be resolved is the use of more than one specimen per test in the temperature gradient trough. Does the investigator have more than one degree of freedom from a single test? The answer is no because it cannot be easily demonstrated that fish in a group act independently of each other. Because fishes are not tagged or tracked during a test to isolate their identity, individual movements usually cannot be determined, particularly over a short interval of time. Only a frequency distribution of temperature occupied is obtained and a measure of its central tendency should be used to indicate preferred temperature. Because Cherry et al. (1975, 1977) had conducted 8 to 18 tests each during rising and falling field temperatures, the degrees of freedom equal the number of tests.

Richards et al. (1977) have expressed the need for standardizing the technique of determining temperature preferences of fishes. As part of that effort it would be helpful to have a standard method of analyzing and interpreting such data. In this spirit, researchers should be encouraged either to provide sufficient information and rationale in publications or to make back-up files available so that the analyses can be reproduced by others. We have proposed a step in that direction in this paper. Only through standardization of experimental and statistical methodology can the questions posed by Richards et al. (1977) be adequately answered.

BARR, A. J., J. H. GOODNIGHT, J. P. SALL, AND J. T. HELWIG. 1976. A user's guide to statistical analysis system. SAS Institute Inc., Raleigh, North Carolina.

CHERRY, D. S., K. L. DICKSON, AND J. CAIRNS JR. 1975. Temperatures selected and avoided by fish at various acclimation temperatures. J. Fish. Res. Board Can. 32: 485–491.

CHERRY, D. S., K. L. DICKSON, J CAIRNS JR., AND J. R. STAUFFER. 1977. Preferred, avoided and lethal temperatures of fish during rising temperature conditions. J. Fish. Res. Board Can. 34: 239–246.

COUTANT, C. C. 1977. Compilation of temperature preference data. J. Fish. Res. Board Can. 34: 739–745.

HALL, L. W. JR , C. H. HOCUTT, AND J. R. STAUFFER JR. 1978. Implication of geographic location on temperature preference of white perch, *Morone americana*. J. Fish. Res. Board Can. 35: 1464–1468.

RICHARDS, F P., W. W. REYNOLDS, AND R. W. MCCAULEY ed.] 1977. Temperature preference studies in environmental impact assessments: an overview with procedural recommendations. J. Fish. Res. Board Can. 34: 729–761.

STAUFFER, J. R. JR., D. S. CHERRY, K. L. DICKSON, AND J. CAIRNS JR. 1975. Laboratory and field temperature preference and avoidance data of fish related to the establishment of standards, p. 119–139. *In* S. B. Saila [ed.] Fisheries and energy production: A symposium. D. C. Heath and Company, Lexington, Mass.

Accumulation of ^{14}C Labeled Benzene and Related Compounds in the Rotifer *Brachionus plicatilis* from Seawater

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ECHEVERRIA, T. 1980. Accumulation of ^{14}C labeled benzene and related compounds in the rotifer *Brachionus plicatilis* from seawater. Can. J. Fish. Aquat. Sci. 37: 738–741.

Rotifers were exposed to (1) a single dose of ^{14}C labeled benzene on day 1 with initial concentrations of benzene at 0.1 and 1.0 μL/L declining over time and (2) a chronic exposure of 0.1 and 1.0 μL/L daily for 9 d. Both conditions resulted in rotifers accumulating 1000 to 10 000 times the ^{14}C activity detected in the seawater. These concentrations were maintained in the rotifers for 8 and 11 d, respectively, and remained high even after exposure was terminated. Analysis of ^{14}C activity from ^{14}C labeled benzene detected in the water revealed its source to be phenolic compounds, compounds with a potentially higher toxicity than benzene. The water maintained a low level of ^{14}C compounds throughout the experiments. Rotifers accumulated high quantities and retained most of the acquired ^{14}C compounds after 2 d in clean water.

Key words: benzene, rotifer, zooplankton, biomagnification

ECHEVERRIA, T. 1980. Accumulation of ^{14}C labeled benzene and related compounds in the rotifer *Brachionus plicatilis* from seawater. Can. J. Fish. Aquat. Sci. 37: 738–741.

Des rotifères furent soumis à (1) une exposition à une seule dose de benzène marqué au ^{14}C au jour 1, de concentrations initiales de benzène de 0,1 et 1,0 μL/L, diminuant avec le temps, et (2) une exposition chronique à 0,1 et 1,0 μL/L quotidiennement pendant 9 jours. Dans les deux situations, il en est résulté chez les rotifères une accumulation de 1 000 à 10 000 fois l'activité du ^{14}C décelée dans l'eau de mer. Ces concentrations se maintiennent chez les rotifères pendant 8 et 11 jours, respectivement, et demeurent à un niveau élevé même une fois l'exposition terminée. L'analyse de l'activité du ^{14}C dans le benzène marqué au ^{14}C décelée dans l'eau montre qu'elle a comme source des composés phénoliques, composés d'une toxicité potentiellement supérieure à celle du benzène. Durant toutes les expériences, l'eau conserve un bas niveau de composés de ^{14}C. Les rotifères accumulent de fortes quantités et conservent la plus grande partie des composée de ^{14}C acquis, après 2 jours en eau propre.

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METABOLISM of petroleum hydrocarbons by animals requires certain NADPH-dependent enzymes (Sims and Grover 1974). Many marine fish and invertebrates are able to metabolize hydrocarbons (Varanasi and Malins 1977). Some marine phytoplankton and invertebrates are unable to metabolize specific hydrocarbons because they do not possess the necessary enzymes. The ability of clams (Neff and Anderson 1975), mussels (Clark and Finley 1975; Lee et al. 1972), barnacles (Morris 1973), oysters (Stegeman and Teal 1973), and jellyfish (Lee 1975) to accumulate oil components, but not to metabolize them, stimulated my interest in biomagnification through the food chain.

The cosmopolitan euryhaline rotifer *Brachionus plicatilis* is easily reared in the laboratory and has proven to be adequate as larval fish food (Theilacker and McMaster 1971). It is now used in the Tiburon Laboratory to rear larval fishes (Struhsaker et al. 1974).

Benzene, a monocyclic aromatic hydrocarbon of the water-soluble fraction of crude oil, has a low toxicity (Hawley 1971) and is soluble in seawater at 1400 μL/L (Benville and Korn 1977). Benzene occurs chronically in the open ocean and in estuaries at about 0.001 to 0.1 μL/L (Lee and Ryan 1976).

To determine the fate of a single component of crude oil in a prey species, I studied the uptake and depuration of benzene in *B. plicatilis* exposed to experimental concentrations of benzene that approximated measured environmental levels.

Methods — A culture of *B. plicatilis* was maintained in an 80-L glass aquarium under constant aeration, illumination (Grolux[1] and fluorescent lights at 215 lx), salinity (20‰ ± 2‰), and temperature (22 ± 2°C). They were fed a 2-μm-long green flagellate (*Nephroselmis* sp.: 10^4 cells/mL) reared under identical conditions, except for a higher light intensity (1615 lx). The rotifers were fed daily for 1 wk before experiments to ensure a healthy population, but not during the experiment.

Double cellulose-filtered (pore size 5 μm) and 8-L single charcoal-filtered seawater was placed in green, 10-L poly-

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[1]Reference to trade names does not imply endorsement by the National Marine Fisheries Service (NOAA).

ethylene pans. The static pans were kept in a water bath at constant light (24 h: 108 lx), temperature (14–16°C), and salinity (23‰). Rotifers were concentrated on a 53-μm Nitex nylon net, rinsed with clean, filtered seawater, and transferred to the experiment pans. Rotifer populations were monitored daily using a model ZBI Coulter counter. Rotifer dry weights were obtained by rinsing with distilled water, drying at 20°C for 24 h, and weighing on a Cahn model 4400 electro-balance (±0.1 μg). The average weight for a rotifer was 0.35 μg. [^{14}C]benzene solution was prepared by combining a benzene stock solution with ^{14}C labeled benzene. The saturated benzene stock solution was prepared by vigorously shaking 99.9% benzene into 1 L of filtered seawater. The concentration was measured on a Tracor MT220 gas chromatograph (GC) (Benville and Korn 1974). An appropriate amount of stock solution was combined with ^{14}C labeled benzene to give the desired specific activity, and then stirred into pans for final concentrations of 0.1 or 1.0 μL/L. [^{14}C]benzene solution activities were monitored by sampling 1 mL from the center of each pan, mixing it with 10 mL Instagel® scintillator, and counting it on a Packard liquid scintillation spectrometer. The activity of experimental seawater was similarly measured daily, before and after the addition of fresh [^{14}C]benzene solution. Initial measurements were used to calculate how much, if any, new [^{14}C]benzene solution is needed to maintain specific activities of 10 and 100 in expt. 1, and 12 and 122 in expt. 2. Water samples were measured on a gas chromatograph (GC) on days 1, 3, and 5 to determine actual amounts of benzene (Fig. 1).

The activity in rotifers was counted by concentrating up to 500 mL of rotifers, or ~0.02 mg on a 53-μm net, washed into a beaker with 10 mL of water, and transferred to a Pasteur capillary pipet plugged at the base with glass wool. Packard-100® solution digestor was added (0.5 mL), and the pipet sealed for 25 h at 20°C. The digest was transferred to a scintillation vial with 10-mL Dimulene® scintillator by using air pressure and then flushing the pipet twice with the scintillator. Samples were counted and corrected for a total method efficiency of 61%, determined by running a known concentration of [^{14}C]benzene through the sampling method.

In expt. 1, rotifers (60/mL) were given a single initial dose on day 1 and left in the dosed pans for 8 d. The contents of two replicate pans for each concentration of benzene, 0.1 and 1.0 μL/L, were monitored daily for ^{14}C activity in the water column and in the rotifers.

In expt. 2, rotifers (20/mL) were exposed to benzene concentrations at relatively constant levels of 0.1 or 1.0 μL/L for 9 d. Because about 70% of the [^{14}C]benzene volatilizes in 24 h (Eldridge and Echeverria 1977), concentrations remaining 24 h after dosing were brought up to the target level by adding [^{14}C]benzene solution equivalent to the amount volatilized. ^{14}C activity in the water was measured daily with the scintillation counter. During the second experiment, the actual concentration of benzene was determined every 2 d with a gas chromatograph for the two replicates of each concentration.

Results — In experiment 1, 80% of ^{14}C was lost from the water to the air, pan surfaces, and rotifers at both initial concentrations of [^{14}C]benzene after 2 d. However, ^{14}C compounds never completely disappeared from the aqueous phase (Fig. 1).

In experiment 2, [^{14}C]benzene was administered to maintain an actual level of ^{14}C activity in the seawater of 0.12 (±0.04 μL/L) and 1.1 (±0.5 μL/L) (Fig. 1) for 9 d. Actual concentration of benzene monitored by the gas chromatograph (GC) was compared to ^{14}C activity monitored by the scintillation counter. On day 1, measurements by both techniques corresponded to the target concentrations of benzene; however, by day 5, the GC failed to detect any benzene in the system while the scintillation counter was showing ^{14}C activity. [^{14}C]benzene solution was added to the experimental pans to maintain the concentration of [^{14}C]-benzene monitored by the scintillation counter. For the duration of these experiments the presence of benzene and of ^{14}C activity were not correlated. Water samples from a pan with a benzene concentration of <0.01 μL/L (GC) and a ^{14}C count corresponding to 0.8 μL/L were sent to an analytical laboratory. Method C (Taras et al. 1971), a standard method for the determination of phenols, indicated that all ^{14}C activity (0.8 μL/L) was due to phenolic compounds. This method includes possible interference from benzene; however, no benzene was detected by GC with a sensitivity of 0.01 μL/L.

Adsorption was measured by taking water samples from the cleaned and refilled pans on day 9, and showed ^{14}C activity of 0.001 μL/L. The only source of ^{14}C would be leaching from the container walls into solution.

In expt. 1, the rotifers accumulated ^{14}C compounds rapidly (Fig. 1), reached an asymptote by day 5, and were maintained for the duration of the experiment. Final biomagnification of ^{14}C compounds in the rotifers on day 8 were 10^2 times, at 0.1 μL/L, and 10^3 times at 1.0 μL/L, the levels in the water. The rotifers accumulated and maintained a high level of ^{14}C compounds which did not appear to be depurated while in the treated water.

In expt. 2, the rotifers under chronic exposure accumulated a maximum of ~10^4 times the initial concentration in the water. A peak in the rotifers was reached on day 6 during which time the benzene (GC) was not present in the water.

Both singly (initial dose) and chronically (constant dose) treated rotifers accumulated ^{14}C compounds to high concentrations. The data suggest that there is a limit to the amount of ^{14}C compounds a rotifer is able to store, with a state of equilibrium being reached between the water and the rotifers. ^{14}C compounds never completely left either system during the 8- or 11-d experimental periods.

Discussion — The presence of phenols in the experiments, instead of benzene, indicates some type of chemical degradation. The stability of benzene and the short duration of these experiments would eliminate photochemical oxidation occurring at any significant level. Possibly the benzene was metabolized by bacteria. Biodegradation of benzene has been demonstrated with in situ marine microbes (Lee and Ryan 1976), specifi-

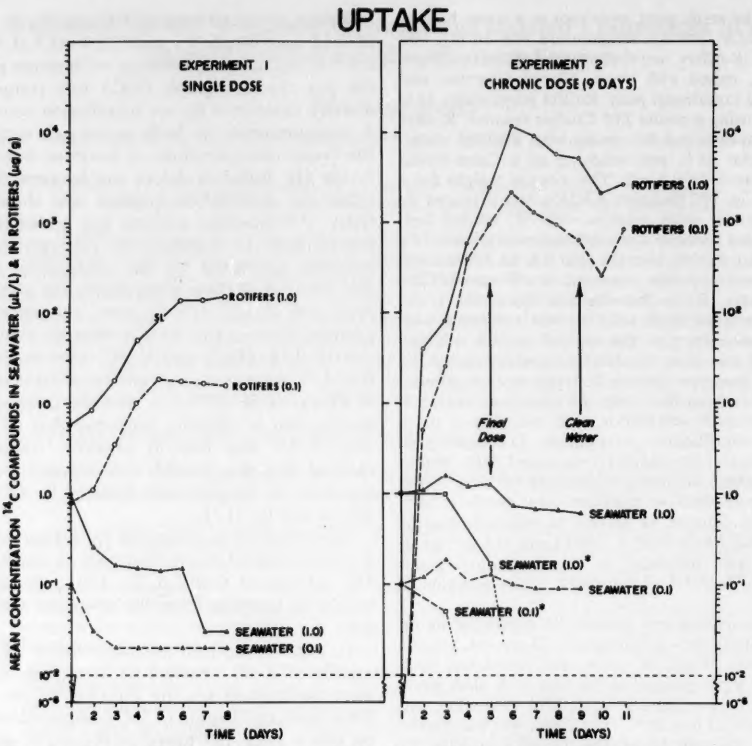

FIG. 1. Expt. 1. Uptake of ^{14}C compounds by the rotifer *B. plicatilis* and the corresponding concentration in the seawater. Expt. 2. Uptake of ^{14}C compounds by *B. plicatilis* under chronic exposure, and the corresponding concentration in the seawater. Values in the seawater measured daily on a scintillation counter and on days 1, 3, and 5 on a gas chromatograph (GC). *GC analysis, concentration of benzene approaches 0 between measurements; solid lines are 1.0 µL/L; broken lines are 0.1 µL/L.

cally the bacteria *Pseudomonas putida* (>1.0 µm long) and *Nocardia* sp. (>1.0 µm long) (Gibson 1976). It is not known whether any marine microbes capable of benzene degradation were present in the experimental pans. To determine if ^{14}C activity was bound to bacteria, a subsample of water from all pans was filtered through 0.45-µm Millipore filter paper and analyzed on the scintillation counter. ^{14}C activity for unfiltered water corresponded to that of the filtered water samples measured on the same day. Therefore, ^{14}C was not bound up in any particle or bacteria larger than 0.45 µm. If the benzene was degraded by bacteria, it was not bound to them.

Biodegradation of benzene by the experimental animals is also a possibility; however, hydrocarbons stored for long periods indicate the inability of animals to metabolize them (Lee 1975). During these experiments, rotifers accumulated and maintained concentrations of benzene and phenolic compounds many times the exposure level. It is possible that the rotifers were metabolizing benzene into phenols, but because ^{14}C compounds were retained by them, the effect is still the same: rotifers accumulated and retained concentrated amounts of ^{14}C compounds during the experimental time frame. Depuration may occur in clean water over a longer time than tested.

Herring larvae exposed to ^{14}C compounds through water accumulated 2 times the concentration in the water, increasing to 3.2 times when fed the rotifer (Eldridge et al. 1978). The ability of the rotifer to accumulate benzene results in the availability of a higher concentration of benzene and related compounds to feeding larval fish, and would increase the possibility of adverse effects. Larval herring showed alterations in their metabolic process when exposed to benzene at 0.02–2.3 µL/L (Eldridge et al. 1977). Juvenile herring assimilate labeled hydrocarbons in the stomach tissue and lipids (Whittle et al. 1977), with unknown conse-

quences. Depuration of hydrocarbons in fish does occur, and depuration of ^{14}C compound in rotifers is an untested possibility. However, rotifers accumulate high concentrations of ^{14}C compounds, larval herring accumulate ^{14}C compounds when feeding on rotifers, and there are metabolic changes in larvae. Just because the fish can metabolize ^{14}C compounds does not eliminate possible adverse effects when both the fish and its food are exposed to the hydrocarbon.

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BENVILLE, P. E. JR., AND S. KORN. 1974. A simple apparatus for metering volatile liquids into water. J. Fish. Res. Board Can. 31: 367–368.

1977. The acute toxicity of six monocyclic aromatic crude oil components to striped bass (*Morone saxatillis*) and bay shrimp (*Crago franciscorum*). Calif. Fish Game 63(4): 204–209.

CLARK, R. C. JR., AND J. S. FINLEY. 1975. Uptake and loss of petroleum hydrocarbons by the mussel, *Mytilus edulis* in laboratory experiments. Fish Res. Bull. 73(3): 508–515 p.

ELDRIDGE, M. B., AND T. ECHEVERRIA. 1977. Fate of ^{14}C benzene (an aromatic hydrocarbon of crude oil) in a simple food chain of rotifers and Pacific herring larvae. Cal-Neva Wildl. Trans. p. 90–96.

ELDRIDGE, M. B., T. ECHEVERRIA, AND J. A. WHIPPLE. 1977. Energetics of Pacific herring (*Clupea harengus pallasi*) embryos and larvae exposed to low concentrations of benzene, a monoaromatic component of crude oil. Trans. Am. Fish. Soc. 106(5): 452–461.

ELDRIDGE, M. B., T. ECHEVERRIA, AND S. KORN. 1978. Fate of ^{14}C benzene in eggs and larvae of Pacific herring (*Clupea harengus pallasi*). J. Fish. Res. Board Can. 35: 861–865.

GIBSON, D. T. 1976. Initial reactions in the bacterial degradation of aromatic hydrocarbons. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1: Orig. Reihe B 162: 157–168.

HAWLEY, G. G. 1971. Condensed chemical dictionary, 8th ed. Van Nostrand Reinhold Co., N.Y.

LEE, R. F. 1975. Fate of petroleum hydrocarbons in marine zooplankton. Proc. 1975 Conference on Prevention and Control of Oil Pollution. Am. Pet. Inst., Washington, D.C. p. 549–553.

LEE, R. F., AND C. RYAN. 1976. Biodegradation of petroleum hydrocarbons by marine microbes, p. 119–125. *In* J. M. Sharpley and A. M. Kaplan [ed.] Proc. of the Third International Biodegradation Symposium. Applied Science Pub., London.

LEE, R. F., R. SAUERHEBER, AND A. A. BENSON. 1972. Petroleum hydrocarbons: uptake and discharge by the marine mussel (*Mytilus edulis*). Science 177: 344–346.

MORRIS, R. J. 1973. Uptake and discharge of petroleum hydrocarbons by barnacles. Mar. Pollut. Bull. 4: 107–109 p.

NEFF, J. M., AND J. W. ANDERSON. 1975. Accumulation, release and distribution of benzo(A)pyrene-^{14}C in the clam (*Rangia cuneata*). Proc. 1975 Conference of Prevention and Control of Oil Pollution. Am. Pet. Inst., Washington, D.C. p. 469–471.

SIMS, P., AND P. L. GROVER. 1974. Polycyclic aromatic hydrocarbons, p. 165–274. *In* D. D. Klein, S. Weinhouse, and A. Haddow [ed.] Advances in cancer research. Vol. 20. Academic Press, New York, N.Y.

STEGEMAN, J. J., AND J. M. TEAL. 1973. Accumulation, release, and retention of petroleum hydrocarbons by the oyster (*Crassastrea virginica*). Mar. Biol. 22: 37–44.

STRUHSAKER, J. W., M. B. ELDRIDGE, AND T. ECHEVERRIA. 1974. Effects of benzene (a water-soluble component of crude oil) on eggs and larvae of Pacific herring and northern anchovy, p. 253–284. *In* J. F. Vernberg and W. B. Vernberg [ed.] Pollution and physiology and marine organisms. Academic Press, Inc., New York, N.Y.

TARAS, M. J., A. E. GREENBERG, R. D. HOAK, AND M. C. RAND [ed.] 1971. Standard methods for the examination of water and wastewater, 13th ed. Washington, D.C.

THEILACKER, G. H., AND M. F. MCMASTER. 1971. Mass culture of the rotifer *Brachionus plicatilis* and its evaluation as a food for larval anchovies. Mar. Biol. (N.Y.) 10(2): 183–188.

VARANASI, U., AND D. C. MALINS. 1977. Metabolism of petroleum hydrocarbons: accumulation and biotransformation in marine organisms, p. 175–270. *In* D. C. Malins [ed.] Effects of petroleum on arctic and subarctic marine environments and organisms: biological effects. Vol. 2. Academic Press, New York, N.Y.

WHITTLE, K. J., J. MURRAY, P. R. MACKIE, R. HARDY, AND J. FARMER. 1977. Fate of hydrocarbons in fish. Rapp. P.-V. Reun. Cons. Int. Explor. Mer 171: 139–142.

Carbon-13/Carbon-12 Variation in Subalpine Lake Aquatic Insects: Food Source Implications

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RAU, G. H. 1980. Carbon-13/carbon-12 variation in subalpine lake aquatic insects: food source implications. Can. J. Fish. Aquat. Sci. 37: 742–746.

Relative ^{13}C concentrations were found to be significantly different among the three primary organic carbon sources for aquatic insect production within Findley Lake, Washington, USA. These three carbon sources were conifer tree detritus (mean $\delta^{13}C = -27.3$ per mil), periphyton (−34.6 per mil), and plankton (−45.9 per mil). Correspondingly, the $\delta^{13}C$ of the adults of assumed autochthonous carbon feeders, *Paraleptophlebia* sp. (Ephemeroptera: Leptophlebiidae) and *Chaoborus trivittatus* (Diptera: Chaoboridae), well approximated the $\delta^{13}C$ of periphyton and plankton, respectively. The remainder of the adult insect emergence, mostly Limnelphilidae and Chironomidae, exhibited $\delta^{13}C$ values intermediate between the terrestrial and periphyton carbon. Approximately 38% of the 51 kg C of insect biomass annually emerging from this lake was conservatively estimated to originate from terrestrial plant sources.

Key words: carbon-13, carbon pathways, lake, aquatic insects, feeding ecology, insect emergence, terrestrial detritus

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Nous avons trouvé des différences significatives de concentration relative de ^{13}C entre les trois principales sources de carbone organique servant à la production d'insectes dans le lac Findley, État de Washington (É.-U.). Ces trois sources de carbone sont: détritus de conifères ($\delta^{13}C$ moyen = −27,3 par mil), périphyton (−34,6 par mil) et plancton (−45,9 par mil). Egalement, le $\delta^{13}C$ d'adultes d'espèces autochtones qu'on suppose se nourrir de carbone, *Paraleptophlebia* sp. (Ephemeroptera: Leptophlebiidae) et *Chaoborus trivittatus* (Diptera: Chaoboridae), se rapproche beaucoup de $\delta^{13}C$ du périphyton et du plancton, respectivement. Le reste de l'émergence d'insectes adultes, Limnelphilidae et Chironomidae en grande partie, accuse des valeurs de $\delta^{13}C$ intermédiaires entre carbone terrestre et carbone périphytonique. On estime d'une manière conservatrice qu'environ 38% des 51 kg de C de biomasse d'insectes émergeant annuellement de ce lac provient de sources végétales terrestres.

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MUCH of the organic carbon present in lake environments can be of terrestrial origin (e.g. Pieczynska 1975; Wetzel 1975). It has also been suggested that upon entering lakes, this land-produced material can become an important carbon and nutrient source for animal production (e.g. Sorokin 1972; Bretschko 1975; Larsson and Tangen 1975; Wetzel 1975; Wissmar et al. 1977). Despite success in demonstrating that some stream invertebrates, in particular insects, can obtain much of their energy and biomass from allochthonous substrates (Hynes 1970; Mann 1975; Anderson and Sedell 1979), a similar linkage between land plants and lake secondary production has generally been less apparent. However, by measuring the natural abundances of stable carbon isotopes in the adult stages of aquatic insects, this study provides a new source of evidence that land-produced detritus can indeed be an important carbon source for some lacustrine animals.

Located within a 104-ha (1 ha = 10 000 m^2) forested watershed, Findley Lake is an example of a small (11.4 ha), deep (28 m), oligotrophic lake common to the subalpine regions of Washington State's Cascade Mountain Range. A large amount of forest detritus is known to enter the lake (Rau 1976), and is rapidly decomposed there (Rau 1978a). Aquatic insects participate in this detrital degradation process (Rau 1978a), and sizable populations of presumed detritus shredders, larval Limnephilidae, are present in the lake (Sherk and Paulson 1973; Rau 1979). These observations led to the hypothesis that a large amount of insect production in Findley Lake is ultimately derived from land plants (Rau 1976; Wissmar et al. 1977). How might such an insect–food relationship be more directly demonstrated?

In an effort to distinguish lake- from land-produced

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organic material, it was discovered that the relative abundance of ^{13}C in Findley Lake plankton was quite unlike that found in terrestrial plants or animals (Rau 1978b). Consequently, the possibility existed that organic material synthesized in the water column of the lake could be isotopically discerned from organic material produced elsewhere in the watershed. This natural isotopic difference between terrestrially and aquatically produced organic carbon could be further used to determine their relative utilization as food by lake animals. The stable carbon isotope method of investigating animal food webs has been previously demonstrated in several terrestrial, estuarine, and marine environments (e.g. DeNiro and Epstein 1978a, b, Fry et al. 1978; Thayer et al. 1978; Boutton et al. 1978; Haines and Montague 1979; Petelle et al. 1979; Rau and Hedges 1979). Little or no difference exists between the carbon isotope ratios of whole animals and that of their food (DeNiro and Epstein 1978a). Thus, in identifying animal food sources, the stable carbon isotope technique involves matching the $^{13}C/^{12}C$ found in the animal's tissues to that of a potential food source or mixture of food sources. To avoid ambiguity, however, it is important that the number of potential carbon/food sources be small and that they be isotopically distinct.

Such conditions are likely present at Findley Lake where land plants, plankton, and periphyton compose the primary sources of lake organic material. Terrestrial plant litter input to the lake is sizable and almost exclusively conifer tree detritus (Rau 1976). A fourth possible carbon source, aquatic macrophytes, was observed to contribute negligibly to the lake's organic carbon budget. Therefore, should the three primary carbon/food sources for lake animals prove to be isotopically distinct, as earlier indicated, such isotopic differences should also be evident among the adults of the various aquatic insect taxa whose larval feeding stages reside within the lake. Such circumstances will be demonstrated.

Material and methods — Adult insects and their primary carbon/food sources were collected for isotope analysis from the extremes of their spatial and/or temporal ranges as well as from places where, and times when, the various insect and food groups were most prevalent. The resulting range of $^{13}C/^{12}C$ within each insect or food group should therefore be somewhat exaggerated over what would be expected from a more random sampling of those materials. Statistically significant differences in $^{13}C/^{12}C$ between food groups and between insect taxa would therefore be indicated should their respective ranges of isotope ratios not overlap.

Adult specimens of the most abundant insect taxa emerging from Findley Lake (Rau 1979) were collected using floating box emergence traps similar to those described by Mundie (1971). A variety of conifer needles and composite forest litter was sampled from the trees and soil surrounding the lake as well as from lake sediments. Periphyton was removed from 30 × 35 cm glass panes which had been suspended vertically above the lake sediments at water depths of 2, 5, and 10 m in the lake. These initially clean glass substrates had been incubated in the lake for a period of 3 mo. Throughout a 1-yr period, plankton was collected from vertical hauls of a plankton net (76-μm mesh) through 25 m of lake water. Most of the chaoborid larvae in these hauls were removed by filtering the samples through a 0.3-mm² mesh screen. Surface sediments from lake depths of 2, 11, and 28 m were sampled by vertically driving into the lake bottom and removing a 3-cm (ID) plastic tube. The top 1 cm of this sediment sample was then extruded from the tube and removed. Upon collection, all of the above samples were dried for several days at 60°C, ground, and stored in a desiccator.

Prior to analysis in a Nuclide 6-60 RMS mass spectrometer, the carbon in each sample was oxidized and purified by methods described by Buchanan and Corcoran (1959) and Rau (1978b). By convention the $^{13}C/^{12}C$ of a sample was reported as the relative per mil difference between the isotope ratio of the sample and that of the PDB carbonate standard (Craig 1957). That is:

$$\delta^{13}C = \{[(^{13}C/^{12}C)_{sample}/(^{13}C/^{12}C)_{PDB}] - 1\}10^3 \text{per mil.}$$

The analytical precision of the $\delta^{13}C$ measurements was $\sim \pm 0.2$ per mil.

Results and discussion — As shown in Fig. 1, the mean $\delta^{13}C$ of the primary organic carbon sources for Findley Lake insects were −27.3 per mil (forest detritus), −34.6 per mil (periphyton), and −45.9 per mil (plankton). The ranges of the $\delta^{13}C$ values within those carbon sources do not overlap despite considerable spatial and temporal heterogeneity of the samples. It is of interest to note that the $\delta^{13}C$ values of the lake's surface sediments are less negative than those of the plankton or periphyton measured (Fig. 1). These elevated sediment $\delta^{13}C$ values, −28.1 to −33.1 per mil (littoral to deep-lake sediments), suggest large inputs of organic carbon relatively rich in ^{13}C. Forest detritus with a mean $\delta^{13}C$ of −27.3 per mil is implicated as such a carbon source. Indeed, a significant contribution of such detritus to the lake (Rau 1976) and ultimately to the sediments (Birch 1976) has been previously observed.

Carbon-13 enrichment relative to autochthonous carbon sources is also evident in the $\delta^{13}C$ measured in a number of adult aquatic insect taxa at Findley Lake (Fig. 1). As in the case of lake sediments, incorporation of land plant carbon into this insect biomass is indicated. In light of DeNiro and Epstein's work (1978a), it is not possible that these elevated insect $\delta^{13}C$ values result from animal isotope fractionation effects operating on the ^{13}C-depleted plankton or periphyton carbon. Rather, dietary supplements of ^{13}C-rich terrestrial detritus likely explain the less negative $\delta^{13}C$ values, −25.2 to −30.8 per mil, found for Chironomidae and the limnephilids, *Lenarchus* sp. and *Halesochila taylori*.

Considering the above three insect taxa, the actual percentage of their carbon biomass that is of terrestrial origin can be roughly approximated. First, a regression line relating food $\delta^{13}C$ to insect $\delta^{13}C$ (Fig. 1, derived from DeNiro and Epstein 1978a) is used to back-calculate the mean food $\delta^{13}C$ that was necessary to produce

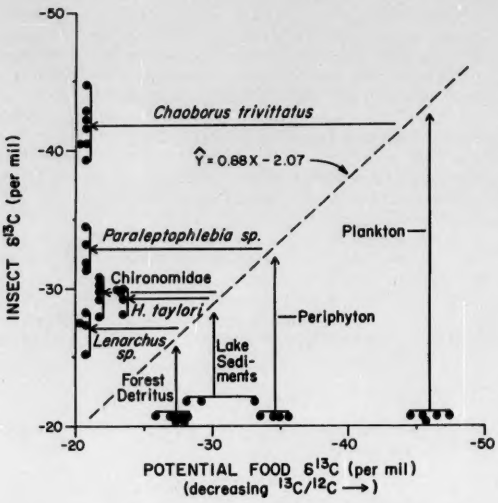

FIG. 1. The $\delta^{13}C$ values and means (arrows) of adult aquatic insects and their primary potential food sources at Findley Lake, Washington. The insect groups analyzed accounted for at least 90% of the total adult biomass annually emerging from the Lake during a 4-yr study (Rau 1979). The average emergence biomass for each insect taxon is listed in Table 1. The broken line represents the linear regression of data reported by DeNiro and Epstein (1978a), 37 analyses of a variety of insects (five families, eight species) relating their $\delta^{13}C$ to that of their known food source under controlled conditions. This regression has an estimation error, $S_{y.x}$, of less than 1.0 per mil.

the observed $\delta^{13}C$ means of Findley Lake insects (Table 1). Then by assuming that these mean food $\delta^{13}C$ estimates result from a mixing of terrestrial and aquatic carbon, represented by forest detritus and periphyton, the proportion of each of these carbon sources in the food (and ultimately in the insects) can be calculated, based on their respective mean $\delta^{13}C$ values using the equation listed in Table 1. If the third possible source of carbon, ^{13}C-depleted plankton (−45.6 per mil), had also been a carbon source for these insects (a not unlikely circumstance, especially in the case of Chironomidae), then the terrestrial carbon percentages listed in Table 1 are underestimates. Underestimation would occur because the autochthonous food $\delta^{13}C$ value used in the calculations should be more negative and is thus overestimated by the periphyton $\delta^{13}C$ mean, −34.6 per mil. Adding up, then, these conservative terrestrial carbon percentages weighted by each taxon's respective mean emergence biomass, at least 38% of the total insect carbon annually emerging from this lake is calculated to originate from terrestrial sources.

In the case of the mayfly, *Paraleptophlebia* sp., its mean $\delta^{13}C$ value well approximates that found for periphyton (Fig. 1). This is not surprising, because many Ephemeroptera have been observed to be periphyton grazers (Merritt and Cummins 1978). On the basis of the $\delta^{13}C$ of these insects at Findley Lake, however, it must be pointed out that similar $\delta^{13}C$ values could be produced if a mixture of high-$\delta^{13}C$-value forest detritus and low-value plankton were being utilized as food. While this possibility may cloud the otherwise probable mayfly–periphyton link, the mayflies' ^{13}C-depleted condition relative to terrestrial car-

TABLE 1. Estimated food $\delta^{13}C$ and percentage of terrestrial carbon in biomass of the quantitatively important aquatic insect taxa at Findley Lake. The calculations are based on the measured $\delta^{13}C$ of the insect and potential food groups using the relationship between insect $\delta^{13}C$ and food $\delta^{13}C$ demonstrated by DeNiro and Epstein (1978a). The estimated percentage of terrestrial carbon in insect biomass is probably conservative. See text for discussion.

Insect taxon	Emergence[a] (kg C·lake^{-1}·yr^{-1})	Mean $\delta^{13}C$[b] (per mil)	Food $\delta^{13}C$[c] (per mil)	Terrestrial carbon[d] (%)
Chaoborus trivittatus	12.7	−41.7	−45.0	(0)
Paraleptophlebia sp.	1.2	−32.4	−34.4	(0)
Chironomidae	5.2	−29.6	−31.2	47
Halesochila taylori	20.8	−29.3	−30.9	51
Lenarchus sp.	7.5	−27.1	−28.4	85
Other insects	3.9	—	—	—
Totals	51.3			38[e]

[a]Annual whole-lake insect emergence. Four-year averages from Rau (1979).
[b]Taxon means from Fig. 1.
[c]Calculated from regression line in Fig. 1. ($S_{y.x}$ <1 per mil).
[d]Percentage of terrestrial carbon = {[food $\delta^{13}C$ − (−34.6)]/[−27.3 − (−34.6)]} × 100, assuming terrestrial food $\delta^{13}C$ = −27.3 and autochthonous food $\delta^{13}C$ = −34.6. See text for discussion.
[e]Sum of percentages weighted by the emergence biomass of each taxon.

bon at least indicates that a significant portion of their carbon biomass must come from nonterrestrial sources.

Chaoborus trivittatus provides an interesting contrast to the species mentioned above. Larvae of these insects were observed to be planktonic and predacious. The guts of the transparent larva were consistently found to be filled with red *Diaptomus* spp. zooplankters. The similarity between the $\delta^{13}C$ of *C. trivittatus* adults and plankton (Fig. 1) further confirms that, unlike the other insects studied, most of the biomass of these animals was derived from the organic carbon found in the lake's water column.

In conclusion, large qualitative variations in the utilization of available carbon/food resources among some Findley Lake aquatic insect taxa are indicated by the sizable differences in whole-body $\delta^{13}C$ of the adult stages of those animals. Furthermore, the elevated $\delta^{13}C$ (relative to the lake's periphyton or plankton) of a number of these insect groups implicates ^{13}C-rich detritus, entering in abundance from the surrounding terrestrial plant community, as an important carbon source for insect production within this lake. As originally suggested by Oana and Deevey (1960), ^{13}C may yet become an important, naturally occurring tracer for the study of carbon biogeochemistry in certain lake and watershed environments.

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ANDERSON, N. H., AND J. R. SEDELL. 1979. Detritus processing by macroinvertebrates in stream ecosystems. Annu. Rev. Entomol. 24: 351–377.

BIRCH, P. B. 1976. The relationship of sedimentation and nutrient cycling to the trophic status of four lakes in the Lake Washington drainage basin. Ph.D. thesis, Univ. Washington, Seattle, Wash. 200 p.

BOUTTON, T. W., G. N. CAMERON, AND B. N. SMITH. 1978. Insect herbivory on C_3 and C_4 grasses. Oecologia 36: 21–32.

BRETSCHKO, G. 1975. Annual benthic biomass distribution in a high mountain lake (Vorderer Finstertaler See, Tyrol, Austria). Int. Ver. Theor. Angew. Limnol. Verh. 19: 1279–1285.

BUCHANAN, D. L., AND B. J. CORCORAN. 1959. Sealed tube combustion for the determination of carbon-14 and total carbon. Anal. Chem. 31: 1635–1638.

CRAIG, H. 1957. Isotopic standards for carbon and oxygen and correction factors for mass-spectrometric analysis of carbon dioxide. Geochim. Cosmochim. Acta 12: 133–149.

DENIRO, M. J., AND S. EPSTEIN. 1978a. Influence of diet on the distribution of carbon isotopes in animals. Geochim. Cosmochim. Acta 42: 495–506.

1978b. Carbon isotopic evidence for different feeding patterns in two hydrax species occupying the same habitat. Science 201: 906–908.

FRY, B., S. JOERN, AND P. L. PARKER. 1978. Grasshopper food web analysis: use of carbon isotope ratios to examine feeding relationships among terrestrial herbivores. Ecology 59: 498–506.

HAINES, E. B., AND C. L. MONTAGUE. 1979. Food sources of estuarine invertebrates analyzed using $^{13}C/^{12}C$ ratios. Ecology 60: 48–56.

HYNES, H. B. N. 1970. The ecology of running waters. Univ. of Toronto Press, Toronto, Ont. 555 p.

LARSSON, P., AND K. TANGEN. 1975. The input and significance of particulate terrestrial organic carbon in a subalpine freshwater ecosystem, p. 351–359. *In* F. F. Wielgolaski [ed.] Ecolological studies. Analysis and synthesis, Vol. 16. Fennoscandia Tundra Ecosystems, Part 1. Springer-Verlag, Berlin.

MANN, K. H. 1975. Patterns of energy flow, p. 248–263. *In* B. A. Whitton [ed.] River ecology. Univ. Calif. Press, Berkeley, Calif.

MERRITT, R. W., AND K. W. CUMMINS. 1978. An introduction to the aquatic insects of North America. Kendall/Hunt, Dubuque, Iowa, 441 p.

MUNDIE, J. H. 1971. Techniques for sampling emerging aquatic insects, p. 80–93. *In* W. T. Edmondson and G. G. Winberg [ed.] A Manual on methods for the assessment of secondary production in freshwaters. Blackwell Scientific, Oxford.

OANA, S., AND E. S. DEEVEY. 1960. Carbon 13 in lake waters, and its possible bearing on paleolimnology. Am. J. Sci. 285-A: 253–272.

PETELLE, M., B. HAINES, AND E. HAINES. 1979. Insect food preferences analysed using $^{13}C/^{12}C$ ratios. Oecologia 38: 159–166.

PIECZYNSKA, E. 1975. Ecological interactions between land and the littoral zones of lakes (Masurian Lakeland, Poland), p. 263–276. *In* G. D. Hasler [ed.] Coupling of land and water systems. Springer-Verlag, New York.

RAU, G. H. 1976. Dispersal of terrestrial plant litter into a subalpine lake. Oikos 27: 153–160.

1978a. Conifer needle processing in a subalpine lake. Limnol. Oceanogr. 23: 356–358.

1978b. Carbon-13 depletion in a subalpine lake: carbon flow implications. Science 201: 901–902.

1979. Carbon sources for aquatic insect production in a subalpine lake. Ph.D. thesis, Univ. Washington, Seattle, Wash. 60 p.

RAU, G. H., AND J. I. HEDGES. 1979. Carbon-13 depletion in a hydrothermal vent mussel: suggestion of a chemosynthetic food source. Science 203: 648–649.

SHERK, T. E., AND D. R. PAULSON. 1973. A progress report on insect emergence at Findley Lake during 1972. Intern. Rep. 17. Coniferous Forest Biome, Seattle, Wash.

SOROKIN, J. I. 1972. Biological productivity of the Rybinsk reservoir, p. 493–503. *In* Z. Kajak and A. Hillbricht-Ilkowska [ed]. Productivity problems of freshwaters. Polish Scientific, Warszawa.

THAYER, G. W., P. L. PARKER, M. W. LACROIX, AND B. FRY. 1978. The stable carbon isotope ratio for some components of an eelgrass, *Zostrea marina*, bed. Oecologia 35: 1–12.

WETZEL, R. G. 1975. Limnology. W. B. Saunders, Philadelphia. 743 p.

WISSMAR, R. C., J. E. RICHEY, AND D. E. SPYRIDAKIS. 1977. The importance of allochthonous particulate carbon pathways in a subalpine lake. J. Fish. Res. Board Can. 34: 1410–1418.

A Standardized Model of Experimental Furunculosis in Rainbow Trout (*Salmo gairdneri*)

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MICHEL, C. 1980. A standardized model of experimental furunculosis in rainbow trout (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci. 37: 746–750.

With the aid of published information, we have developed a standardized and reproducible experimental model of furunculosis in rainbow trout (*Salmo gairdneri*). The infective doses (LD_{50} = 200 to 2000 germs, i.m.) employed strains of *Aeromonas salmonicida*, the virulence of which was maintained by passage in 15-g fingerlings. The number of viable cells in the dose was conveniently determined using the drop-plate enumeration technique; however, meaningful cell counts could only be obtained if broth cultures used for infecting the fish were harvested early in the exponential growth phase (OD < 1.000 at 525 nm). Better results were obtained with intramuscular injection than with intraperitoneal injection. The infection procedure involved injecting a dose of 10 LD_{50}, intramuscularly, into each of 30 fish held at 15°C and recording the mortalities for 10 d. Protection tests in which Tribrissen (28 mg sulfadiazine and 5.6 mg trimethoprim/kg fish for 8 d) was fed or tetracycline (1 mg per fish) i.m. injected into the infected fish served to demonstrate the value of the model. Results were in agreement with field observations with no death for treated fish and a mortality of 96% for untreated fish.

Key words: rainbow trout, *Salmo gairdneri*; *Aeromonas salmonicida*, furunculosis, experimental infection

MICHEL, C. 1980. A standardized model of experimental furunculosis in rainbow trout (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci. 37: 746–750.

En tenant compte de données déjà publiées, nous avons mis au point un modèle de furonculose expérimentale reproductible et standardisé sur truites arc-en-ciel. Des souches de *Aeromonas salmonicida* de haute virulence (DL_{50} = 200 à 2000 germes i.m.) ont été entretenues par passages successifs sur truitelles de 15 g. Une technique de numération en gouttes a permis de surmonter les difficultés liées à l'auto-agglutination des bactéries. La maîtrise des doses inoculées découle des propriétés de croissance de *A. salmonicida* et nécessite une récolte des cultures en phase exponentielle précoce (DO < 1,000 à 525 nm). La virulence dépend de la voie d'administration, l'injection intramusculaire étant très supérieure à l'injection intrapéritonéale. Ces résultats ont permis d'établir un protocole standard par injection i.m. d'environ 10 DL_{50} sur des lots de 30 poissons, les conditions ambiantes étant définies (température de l'eau = 15°C). Les mortalités sont suivies sur 10 jours. Des essais de protection par administration orale de Tribrissen (28 mg sulfadiazine et 5,6 mg trimethoprime/kg de poisson pendant 8 jours) ou injection i.m. de tetracycline (1 mg par poisson) ont permis de contrôler la valeur du modèle. Les résultats recoupent les observations de terrain (pas de mort chez les animaux traités; 96% de morts chez les témoins).

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A great deal of work has been done on *Aeromonas salmonicida*, the specific agent of furunculosis in fish, to study various features of the disease: conditions of occurrence, transmission and course of the infection, factors of pathogenicity, mechanisms of host's defences, efficacy of chemotherapy, or active immunization. In spite of significant results, progress in developing effective control methods for the disease are greatly handicapped by the lack of a satisfactory and reproducible method for experimentally reproducing the disease. Various methods for infecting fish are described

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in the literature (Clayton 1927; Duff 1942; Spence et al. 1965; Amend 1969; Bullock et al. 1976) but a generally acceptable system has yet to be proposed. Two kinds of problems are mainly encountered: first, to select virulent strains and produce the disease with a small number of bacteria, and second, to control the quantity of administered cells and to obtain a wanted effect. The purpose of this study was to solve these problems and develop a standard method for infecting fish with *A. salmonicida* — a method that we believe closely simulates conditions occurring naturally. To do this we have restricted ourselves to using (1) small and controlled numbers of pathogens, (2) the percutaneous route of injection, and (3) a method susceptible to prevention by chemotherapy as in natural infections.

Material and methods — The work was carried out on rainbow trout (*Salmo gairdneri*) of 10–15 g, purchased from hatcheries with a furunculosis-free history, and reared in tanks supplied with dechlorinated recirculating freshwater. They were allowed to acclimatize for at least a fortnight, during which time they were inspected for undesirable signs and routinely bathed in formalin to prevent parasitism. Dry commercial pellets were fed daily. Some individual sera were taken and tested to verify that anti-*A. salmonicida* agglutinins were absent. The modified microtitration method of Paterson and Fryer (Maisse and Dorson 1976) was used for this purpose.

When perfected, the system was tested on fish of different origin or different size (10–100 g), and on a batch of brown trout (*S. trutta*) averaging 6 g. Experimental lots of fish were held in independant 12-L aquaria receiving 15°C tap water. The influence of temperature on the course of furunculosis has been well documented (Mackie et al. 1933; Groberg et al. 1978); a water temperature of 14–15°C favors the disease. Each aquarium was stocked with 10 fish for the median lethal dose (LD_{50}) determinations, or with 30 fish for the chemotherapy efficacy tests. Some factors, such as water quality, oxygenation, flow, and density of fish, are of importance, but were not easy to control in our facilities. To avoid a risk of systematic error due to their effects, we allocated the various fish treatments (dose size or antibiotic used) randomly among the aquaria.

Three strains of *A. salmonicida*, all isolated from clinical cases, were studied. To maintain or increase their virulence, we subcultured them in trout by injecting infected kidney tissues (0.1 mL) from dead trout intramuscularly into healthy trout. Strains were selfagglutinating. One of them was available in a nonaggregating form, but several trials to infect fish with this form proved unsuccessful.

To produce virulent *A. salmonicida* suspensions, we first isolated the bacteria from the kidneys of infected trout on trypticase soy agar (TSA) plates incubated for 48 h at 22°C. A single colony was suspended in 7 mL of tryptic soy broth (TSB) at the same temperature, and after 24 h, the broth culture was used to inoculate the production medium (100 mL of TSB contained in an Erlenmeyer flask). The latter was incubated at 22°C on a magnetic stirrer and cells were harvested at a preselected turbidity, read at 525 nm on a BECKMAN 24 spectrophotometer. The culture was diluted to the desired concentration for infection and 10-fold serial dilutions in physiological saline were prepared and titrated for viable cells using the drop-plate technique of Miles and Misra (1938).

The initial experiments were conducted to compare the virulence of a strain of *A. salmonicida* using two methods of infection: intramuscular (i.m.) and intraperitoneal (i.p.) injection. Fish were injected via i.m. or i.p. routes with 10-fold serial dilutions of the titrated bacterial suspension (10 fish per dose) and the ensuing mortalities recorded for each dose. LD_{50} values were then estimated by the moving averages method of Meynell and Meynell (1970). All injections were administered in a volume of 0.1 mL with control fish receiving saline instead of the bacterial suspension. Prior to injection, the fish were anesthetized with 50 mg/L of MS 222 (Sandoz). Kidneys of all fish dying without typical lesions ("furuncles") were cultured to verify the presence of *A. salmonicida*. The experiments were terminated on the 10th d postinfection at which time mortalities were summed. The chi-square test was used to determine the statistical significance of the results.

Subsequent infection experiments were conducted to investigate the ability of certain antibiotics to arrest the infections. In these experiments i.m. doses of about 10 LD_{50} were used to establish the infections. Fish averaging 12 g were divided into three groups of 30 animals and an additional group of 10. The first group of 30 fish was fed for 8 d with a sulfadiazine–trimethoprim (Tribrissen) supplemented diet. Daily doses were, respectively, 28 mg sulfadiazine and 5.6 mg trimethoprim/kg fish. The second group of 30 fish received a single injection of tetracycline (1 mg per fish) i.m. The selection of these therapeutics was based on earlier in vitro sensitivity testing with *A. salmonicida*. Experimental infection was performed on the 4th d of feeding, and on the same day as tetracycline administration, and consisted of an i.m. injection of 1500 bacteria (strain TG 72/78 "R," $LD_{50} \simeq 150$ cells per fish). The third lot of 30 fish was infected and held as an untreated control, and the fourth group of 10 fish served as a noninfected control. All animals received two injections, physiological saline replacing bacteria or tetracycline where appropriate.

Results — Serial passages of *A. salmonicida* in fingerlings resulted in an increase of virulence. Strains which required large doses (about 10^5 to 10^6 cells) to reproduce the disease just after their isolation or their culture on artificial media reached an LD_{50} between 200 and 2000 germs via an i.m. injection.

Growth curves for *A. salmonicida* in TSB were characterized by fast growing, due to the stirring and oxygenation of the medium, and consistently showed a peculiar phenomenon. After some hours of exponential growth, a sudden break occurred in the curves (Fig. 1). From this point onwards, it was impossible to relate bacterial mass (measured in terms of OD) to the number of viable cells. The reason for the phenomenon is unknown but to obtain standardized and suitable microbial suspensions, it was necessary to harvest the cells during the early phase of exponential growth, namely, before the viable cell counts reached 1.5×10^8 cells/mL.

Figure 2 shows the type of cumulative mortality curves obtained with increasing doses of a virulent strain (TG 59/77 "R") injected intramuscularly or intraperitoneally. In animals receiving i.m. inoculation, the LD_{50} appeared to be below 2000 bacteria and the

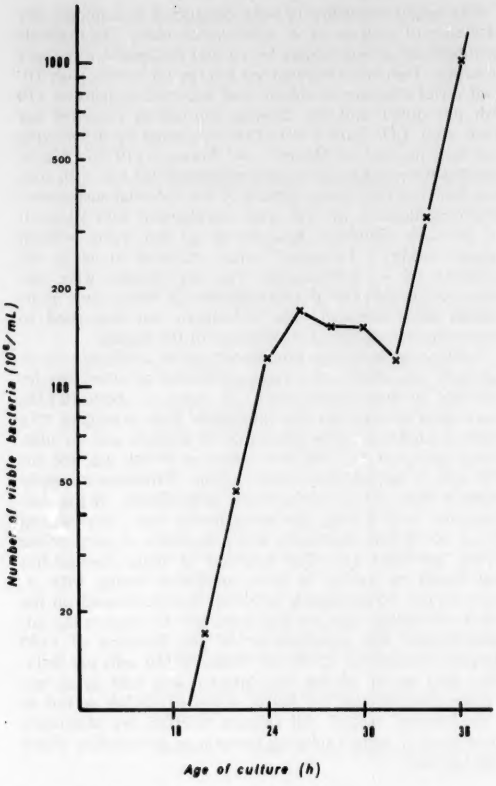

FIG. 1. Logarithmic graph representation of exponential phase of *Aeromonas salmonicida* growth, using the enumeration of viable cells (TSB stirred culture at 22°C; inoculum ≃1000 bacteria/mL).

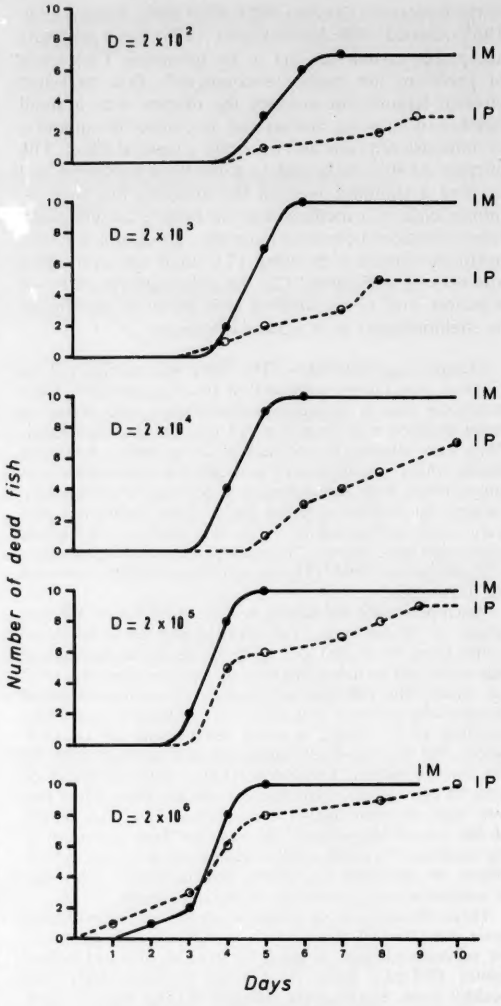

FIG. 2. Cumulative mortality curves obtained in groups of rainbow trout fingerlings held in freshwater at 15°C, injected intramuscularly (i.m.) or intraperitoneally (i.p.) with increasing numbers of bacteria per fish (D) of a virulent strain of *A. salmonicida*.

mortality curves were constantly S-shaped. The animals developed a typical furuncle at the site of injection, and septicemia led to their deaths. The i.p. route resulted in a slower and more irregular mortality response, and higher doses were apparently required to effect a given kill. Typical furuncles were rarely observed on skin. Hemorrhages in the internal organs were a constant feature but these were not pathognomonic, and isolation of the pathogen was therefore needed to confirm furunculosis. To determine whether i.m. and i.p. injections produced different results, we compared the mortalities on the 10th d, for the two lowest doses. A significant difference was found only in groups injected with 2000 cells ($P < 0.05$). It must be borne in mind that small numbers of fish in each group reduce the discriminating value of tests, and this difference indicates a real superiority of the i.m. route.

Table 1 gives results of experimental infection in treated or untreated groups. Protection seems as effective with orally given Tribrissen as with i.m. tetracycline administration. No fish died in treated aquaria, while controls were all killed by the infection. We can consequently control our experimental furunculosis by oral treatment, as fish culturists do in natural outbreaks of the disease.

Table 2 lists some experiments performed with the

TABLE 1. Comparative mortalities of 15-g rainbow trout fingerlings experimentally infected with *Aeromonas salmonicida*, after or without antibiotic protection (strain TG 72/78 "R," $LD_{50} \simeq 150$ bacteria per fish i.m.).

Group	Number of fish	Antibiotic treatment	Challenge (day 0)	Mortalities at 10th d
1	30	Sulfadiazine–trimethoprim 28 mg–5.6 mg/kg fish per day, orally (days −4 to +4)	1500 bacteria (i.m.)	0
2	30	Tetracycline (single injection i.m.: 1 mg per fish) (day 0)	1500 bacteria (i.m.)	0
3 (Controls)	29[a]	0	1500 bacteria (i.m.)	28
4 (Uninfected controls)	10	0	0	0

[a]One fish died by accident just after inoculation.

model over a period of 5 mo. Evaluation of infective doses from OD and actual injected numbers of bacteria are compared. Fish of various origin and size were used, and the observed mortalities always conformed to what we expected. The schedule was applied to brown trout (*S. trutta*) with good results, but we did not compare other ways of infection in this species.

Discussion — The use of virulent cultures enables the establishment of furunculosis in trout with small doses (about 500 cells per fish) of *A. salmonicida*. It avoids the unrealistic loading of the fish's system with high numbers of bacterial cells that is necessitated when low-virulence strains are used, and thus makes it easier to detect multiplication of the pathogen in the host. In fact, we found difficulties in early attempts at infecting animals, due to a loss of pathogenicity of the strains resulting from storage and growth on artificial media. This pathogenicity could be restored by subcultivation in fish. Such in vivo passages have also been successfully practised by Groberg et al. (1978). The same method was used for maintenance of our virulent strains since it had been noticed from previous attempts that, if freezing at −70°C conserved the pathogenic properties well, the titer was reduced after 4 mo.

In agreement with Udey and Fryer (1978) we observed that only autoagglutinating strains were highly virulent. The colonies exhibited the following features: self-aggregation in physiological serum, sedimentation at the bottom of broth that had not been stirred, greyish appearance in a 45° transmitted light, and staining by the crystal violet test of White and Wilson (1951).

In reproducing furunculosis, the efficacy of a parenteral injection has already been stated (Groberg et al. 1978). Moreover, it lets us know the number of bacteria that actually penetrates the organism (this is not the case with balneation). The i.m. route was superior to the i.p. route, at least in rainbow trout, because it was more effective, it simulated a typical form of furunculosis (Klontz et al. 1966), and the regular shape of the mortality curves was of interest in the mathematical analysis of the results. Therefore, we selected this route to standardize the technique. There is likely some leakage of infective material when injecting fish intramuscularly, but the use of convenient

TABLE 2. Control of injected doses of *A. salmonicida*: differences between estimated and checked values, in several experiments performed with rainbow trout of various origin.

Date	Fish weight (g)	Estimated doses (from turbidity)	Injected doses (plate enumeration)	Differences (%)	Mortality (% of fish)
March 17, 1979	70	10 300	10 560	+2.5	100
April 6, 1979	70	5 000	5 200	+4	[a]
	70	3 600	2 800	−22	76.5
	70	1 000	960	−4	41.5
June 13, 1979	85	10 000	11 200	+12	75
June 27, 1979	70	60 000	59 000	−1.5	66
July 3, 1979	8	1 000	960	−4	73
	8	1 000	720	−28	50

[a]Not recorded.

and standardized syringes and needles reduces its importance, and the error is constant.

In developing a highly reproducible experimental disease, we had to minimize or eliminate more serious causes of variability. These stemmed from three main sources: environment, pathogen, and host. Random distribution of treatments among aquaria was sufficient to mitigate the influence of environmental conditions. Control of infective doses has been found to depend upon the age of the culture. If harvested late, the suspension will provide unpredictable results, but when harvested earlier, it is possible to adjust the quantity of inoculated bacteria using a relation curve between OD and viable cells, and to obtain coherent and reproducible results (Table 2). Nevertheless, such a reproducibility is limited to a given lot of trout. The susceptibility of salmonids to furunculosis varies with their size and species, and fish of definite size are not always available when needed. In addition, fish farm practices encourage genetic heterogeneity rather than homogeneity in trout. These factors make reproducibility of the method difficult. Hence we performed an LD_{50} determination on the test fish shortly before using them, so that the final selection of an appropriate infective dose was reasonably well established.

Taking this into account, we could verify the value of our standard using rainbow trout of different sizes, and even a batch of brown trout (we seldom work on this species because healthy, *A. salmonicida*-free specimens are very difficult to obtain). Finally, the model can be applied to different kinds of fish. It simulates a natural subacute infection, and drugs used to combat the natural infection also arrest the experimental infection (Amend 1969, McCarthy et al. 1974). Results are obtained within only 10 d and the statistical analysis is greatly facilitated by the regularity of the mortality curves. We have found that doses of 10 LD_{50} which kill almost all of the untreated animals are ideal for demonstrating the in vivo protective effect of antibiotics. We visualize that the technique will also find application in investigating the efficacy of other products such as antifurunculosis vaccines.

Acknowledgments — This work was supported in part by the Conseil Supérieur de la Pêche.

I am very grateful to Dr T. P. T. Evelyn, Pacific Biological Station, Nanaimo, for his helpful criticisms and painstaking improvement of the manuscript, and to Danièle Potier and Bernadette Fourbet for their technical assistance.

AMEND, D. F. 1969. Oxytetracycline efficacy as a treatment for furunculosis in coho salmon. U.S. Dept. Interior, Bur. Sport Fish. Wildl. Tech. Pap. 31: 6 p.

BULLOCK, G. L., H. M. STUCKEY, R. L. HERMAN, AND C. E. SMITH. 1976. Comparative susceptibility of Atlantic salmon (*Salmo salar*) to the enteric redmouth bacterium and *Aeromonas salmonicida*. J. Wildl. Dis. 12: 376–379.

CLAYTON, F. H. A. 1927. Notes on salmon furunculosis. Rep. Dove Mar. Lab., Cullercoats, N.S. 16: 49–55 p.

DUFF, D. C. B. 1942. The oral immunization of trout against *Bacterium salmonicida* J. Immunol. 44(1): 87–94.

GROBERG, W. J., R. H. MCCOY, K. S. PILCHER, AND J. L. FRYER. 1978. Relation of water temperature to infections of coho salmon (*Oncorhynchus kisutch*), chinook salmon (*O. tshawytscha*), and steelhead trout (*Salmo gairdneri*) with *Aeromonas salmonicida* and *A. hydrophila*. J. Fish. Res. Board Can. 35: 1–7.

KLONTZ, G. W., W. T. YASUTAKE, AND A. J. ROSS. 1966. Bacterial diseases of the Salmonidae in the Western United States: pathogenesis of furunculosis in rainbow trout. Am. J. Vet. Res. 27(120): 1455–1460.

MACKIE, T. J., J. A. ARKWHRIGHT, T. E. PRYCE-TANNATT, J. C. MOTTRAM, W. D. JOHNSTON, W. J. M. MENZIES, AND FURUNCULOSIS COMMITTEE. 1933. Second interim report of the Furunculosis committee. HM Stationery Off. Edinburgh. 81 p.

MAISSE, G., AND M. DORSON. 1976. Production d'agglutinines anti-*Aeromonas salmonicida* par la Truite arc-en-ciel adulte: influence de la température; d'un adjuvant et d'un immunodépresseur. Ann. Rech. Vet. 7(4): 307–313.

MCCARTHY, D. M., J. P. STEVENSON, AND A. W. SALSBURY. 1974. Combined *in vitro* activity of trimethoprim and sulphonamides on fish-pathogenic bacteria. Aquaculture 3: 87–91.

MEYNELL, G. G., AND E. MEYNELL. 1970. Theory and practice in experimental bacteriology. 2nd ed. Cambridge Univ. Press, New York, N.Y.

MILES, A. A., AND S. S. MISRA. 1938. The estimation of the bacteriocidal power of the blood. J. Hyg. 38: 732–749.

SPENCE, K. D., J. L. FRYER, AND K. S. PILCHER. Active and passive immunization of certain salmonid fishes against *Aeromonas salmonicida*. Can. J. Microbiol. 11: 397–405.

UDEY, L. R., AND J. L. FRYER. 1978. Immunization of fish with bacterins of *Aeromonas salmonicida*. Mar. Fish. Rev. 40(3): 12–17.

WHITE, P. G., AND J. B. WILSON. 1951. Differentiation of smooth and nonsmooth colonies of brucellae. J. Bacteriol. 61: 239–240.

BOOK REVIEWS/COMPTES RENDUS

Book review editor:
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Advances in Aquaculture. *Edited by T. V. R. Pillay and W. A. Dill. Fishing News Books Ltd., Farnham, Surrey, England. 1979. xviii, 653 p. £52.*

The 1976 FAO Technical Conference on Aquaculture held in Kyoto, Japan was the largest fisheries conference ever organized by FAO, and a landmark not only for aquaculture but for fisheries as a whole. Its success was due in no small part to the sudden and widespread awareness that few fisheries ecosystems could still be considered as self-sustaining. Traditional models for conserving fisheries resources were failing time and time again, so the aquaculture sector became recognized as a major source of ideas and of alternative approaches to the care and husbandry of fisheries resources.

"Advances in Aquaculture," ably edited by Drs Pillay and Dill and elegantly published by Fishing News Books Ltd., presents the review and experience papers prepared for that Conference. The 117 papers by 178 contributors display not only the progress, but also the technology, the institutional structures, the scientific approaches, and the socioeconomic relevance of these aquatic forms of food production. Specifically, they address world aquaculture and its future role; finfish culture in ponds; culture of crustaceans, molluscs, algae, and seaweeds; aquaculture in raceways, cages, and enclosures; wastes and use of recirculating water in aquaculture; artificial recruitment and transplants; nutritional requirements and feed technology; and genetics and genetic improvement of fish.

Readers will note an emphasis on the concepts or scientific principles of husbandry applicable to a wide variety of site specific situations, also an emphasis on applied systems approaches for the use of water resources, feed, fertilizer, and seed stocks as instruments of rural development. The book provides a ready reference to the sciences basic to the husbandry of fisheries resources and a measure of their present application, enabling an easy comparison with scientific practices in the other renewable resource sectors. Moreover, it provides models for practical and intensive use of specific areas of the world where some form of tenure can be practiced, which in all likelihood pioneer the concepts of resource husbandry that will be applied more widely to extensive regions or economic zones in the future. One is struck by the fact that the routine surveying and monitoring of environmental parameters and of natural fish stocks which have taken up such a large part of the fisheries scientific efforts within developed countries in the past decade are no longer of great interest, and that the focus is clearly turning to the systematic and designed experimental approaches needed to bring forth new knowledge upon which the future changes in food production through fisheries must be based.

Aquaculture topics now constitute a significant proportion of the current world's fisheries literature. This comprehensive and analytical book on such an important segment of fisheries is recommended reading for planners concerned with holistic use of a resource base on either a micro or macro scale, for investors who wish to weigh risks associated with the unknown, for educators wishing to remain relevant to future needs of society, and for the practitioners who wish to refine or supplement their production systems. Equally important, it is commended to the growing body of fisheries scientists who worry about the obsolescence of their research approaches, and wish to ensure their relevance to future fisheries production in both the developed and developing countries of the world.

G. I. PRITCHARD

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(R294)

Books received/Livres reçus[1]

October/octobre 1979

Exotic Species in Mariculture. *Edited by Roger Mann. MIT Press, Cambridge, Mass. 1979. xii + 363 p. $21.00.* (This volume is the proceedings of a Symposium on Exotic Species in Mariculture: Case Histories of the Japanese Oyster, *Crassostrea gigas* (Thunberg), with implications for other fisheries, held at Woods Hole Oceanographic Institution, Woods Hole, Massachusetts, September 18–20, 1978. It contains 17 papers, a Preface, and one Appendix.)

***Lake Restoration. Proceedings of a National Conference.** *August 22–24, 1978, at Minneapolis, Minn. Anonymous editor. U.S. Environmental Protection Agency, Washington, D.C. 1979. viii + 254 p. $4.50.* (This volume, containing 33 papers, an opening address, and three appendices, is available from the U.S. Government Printing Office, Washington, D.C. 20402, as GPO Stock Number 055-001-01-84-2/EPA 440/5-79-001.)

***The Cyclostomata. An Annotated Bibliography, Supplement 1973–1978.** *Dr. W. Junk BV Publishers, The Hague, The Netherlands. 1979. xii + 296 p. $78.95.* (This volume supplements the original work published in 1977; it contains another 1081 references to add to the original 4535.)

***Getting Your Start in Flyrod Fishing.** *Keith C. Schuyler. David McKay Book Co., Inc., New York, N.Y. 1979. xvi + 112 p. $12.95.* (A beginner's primer on fly-fishing.)

***Marine, Municipal and Industrial Wastewater Disposal.** *Executive Editor S. H. Jenkins. Pergamon Press, Oxford, New York. 1979. ix + 291 p. $42.00.* (This is Volume 4 of Progress in Water Technology, and consists of the Proceedings of a Conference on Marine, Municipal and Industrial Wastewater Disposal, held in Sorrento, Italy, June 23–27, 1975. It contains 21 papers.)

Descriptive Physical Oceanography. An Introduction. *3rd Edition. G. L. Pickard. Pergamon Press, Oxford, New York. 1979. ix + 233 p. $9.95 (Flexicover).* (According to the author's Preface, this 3rd edition endeavors to update the presentations of the topics in earlier editions, and adds brief descriptions of some new features, such as ocean eddies, without substantially adding to the length of the text.)

***Advances in Aquaculture.** *T. V. R. Pillay and W. A. Dill. Fishing News Books Ltd., Farnham, Surrey, England. 1979. xviii + 653 p. £52.00.* (See review this issue.)

Seawater Aquariums. The Captive Environment. *Stephen Spotte. John Wiley & Sons, New York, NY. 1979. xxii + 413 p. $21.50.* (The author has aimed at producing a soundly based, practical manual of seawater aquariology, from long personal experience.)

November/novembre 1979

***Polycyclic Aromatic Hydrocarbons in the Aquatic Environment. Sources, Fates and Biological Effects.** *Jerry M. Neff. Applied Science Publishers Ltd., London, England. 1979. xii + 262 p. £50.00.* (This relatively short book constitutes a major review of the subjects described in the title.)

***A History of Refrigeration throughout the World.** *Roger Thévenot (English translation by J. C. Fidler). International Institute of Refrigeration, Paris, France. 1979. 476 p.* (Although much of this volume is probably irrelevant for readers of this Journal, the various sections on applications of refrigeration to fish and fisheries may be of some interest.)

***The Assessment of Sublethal Effects of Pollutants in the Sea.** *A Royal Society Discussion, May 24–25, 1978. Organized by H. A. Cole. The Royal Society of London, London, England. 1979. viii + 235 p. £18.50 (£19.10 overseas).* (The papers presented in this Discussion were first published in Philosophical Transactions of the Royal Society of London, Series B, volume 284 (no. 1015), pages 397–633. Thirteen papers, each with its own separate discussion by various participants, comprise the present work.)

Foreign Investment in the U.S. Fishing Industry. *Jeremiah J. Sullivan and Per O. Heggelund. Lexington Books, D. C. Heath and Co., Lexington, Mass., and Toronto, Canada, xiv + 185 p. $21.00 Can.* (This is the third in this publisher's Pacific Rim Series on the Pacific community. It especially concentrates on the present and potential impacts of foreign investment, particularly Japanese.)

December/décembre 1979

Environmental Atlas of the Potomac Estuary. *A. J. Lippson, M. S. Haire, A. F. Holland, F. Jacobs, J. Jensen, R. L. Moran-Johnson, T. T. Polgar, and W. R. Richkus. Martin Marietta Environmental Center Publication, Baltimore, MD. 1979. 280 p. + 9 folio Maps. $19.00.* (This Atlas was prepared by the Environmental Center of the Martin Marietta Corporation for the Power Plant Siting Program, Maryland Department of Natural Resources. Although the Atlas specifically refers to the Potomac Estuary, most of the physical and biological processes described are common to all temperate zone estuaries. The large (10″ × 12″ × ⅝″), soft-covered book and the folio maps are supplied in a hard case, and may be ordered through the Power Plant Siting Program, Maryland Dept. of Natural Resources, Annapolis, Maryland 21401.)

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[1]Entries in this list *not* marked by an asterisk are available for review by interested readers for a 3-month period from the time of publication of the list. For information, please write to the Book Review Editor.

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ANNOUNCEMENTS/AVIS

Third International Congress on the History of Oceanography

The Third International Congress on the History of Oceanography will be held at Woods Hole, MA, USA, September 22–26, 1980. It will be followed by an Assembly, September 29–October 2, entitled "Will we use the oceans wisely? The next 50 years in oceanography." These events are planned as integral parts of the celebration of the 50th Anniversary of the Woods Hole Oceanographic Institution.

The Congress will open with addresses by Drs Paul M. Fye and Roger Revelle and Sir George Deacon. There will be morning symposia on the histories of American Oceanography, Instrumentation, Pollution, and Plate Tectonics. The afternoon sessions will include some 55 papers representing contributions from 18 countries. There will also be special exhibitions and evening lectures.

The Assembly will consist of morning and afternoon sessions in which invited speakers will present papers on the current status and future trends of all aspects of marine science and policy. The sessions will cover scientific problems on the small and local scales, the oceanic regional scale, the global scale, and the human scale. Each session will conclude with a panel paper and discussion on important policy questions raised by the preceding subject matter.

For further information about these meetings, write to *Dr John H. Steele, Director, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA.*

Troisième Congrès international sur l'histoire de l'océanographie

Le Troisième congrès international sur l'histoire de l'océanographie se tiendra à Woods Hole, MA, É.-U., du 22 au 26 septembre 1980. Il sera suivi d'une assemblée, du 29 septembre au 2 octobre, dont le sujet a pour titre : « Utiliserons-nous les océans avec discernement? Les 50 prochaines années en océanographie ». Ces événements font partie intégrale des cérémonies marquant le 50e anniversaire de la Woods Hole Oceanographic Institution.

Le congrès débutera avec allocutions par les Drs Paul M. Fye et Roger Revelle, et Sir George Deacon. Il y aura colloques d'avant-midi sur l'histoire de l'océanographie américaine, de l'instrumentation, de la pollution et de la tectonique des plaques. Les séances d'après-midi incluront quelques 55 communications, contributions de 18 pays. Il y aura aussi expositions spéciales et conférences du soir.

L'assemblée consistera en séances d'avant et d'après-midi, au cours desquelles des orateurs invités présenteront des communications sur l'état actuel et les tendances futures de tous les aspects de la science et de la politique de la mer. Les séances porteront sur des problèmes scientifiques à échelles petites et locales, échelle régionale océanique, échelle globale et enfin, échelle humaine. Chaque séance se terminera par une présentation du groupe d'experts et discussion sur d'importantes questions de politique soulevées par le sujet traité antérieurement.

Pour plus amples renseignements sur ces réunions, on est prié d'écrire au *Dr John H. Steele, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA.*

Joint Oceanographic Assembly, 1982

Canada will host the Fifth Joint Oceanographic Assembly (JOA) in the summer of 1982. It will be held August 2–13 at Dalhousie University, Halifax, Nova Scotia. A national organizing committee with representatives from Dalhousie University, McGill University, the National Research Council of Canada, the Federal Department of Fisheries and Oceans, and the Province of Nova Scotia will attend to local arrangements.

The scientific program will consist of selected topics of broad interdisciplinary interest in marine science. The selection of subjects for the symposia will be the responsibility of a Scientific Program Committee appointed by the Executive of the Scientific Committee on Oceanic Research (SCOR) of the International Council of Scientific Unions (ICSU).

Further information regarding the scientific program and local arrangements for the Assembly will be issued in due course — *Leo O'Quinn, Executive Secretary, National Steering Committee for JOA, c/o Canadian Committee on Oceanography, 240 Sparks Street, Ottawa, Ontario, Canada K1A 0E6.*

Assemblée mixte sur l'océanographie, 1982

C'est au Canada que se tiendra la 5e Assemblée mixte sur l'océanographie, à l'été de 1982. La session aura lieu du 2 au 13 août à l'Université Dalhousie, Halifax, Nouvelle-Écosse. Les arrangements locaux seront par un comité national d'organisation composé de représentants de l'Université Dalhousie, de l'Université McGill, du Conseil national de recherches du Canada, du ministère fédéral des Pêches et des Océans et de la Province de la Nouvelle-Écosse.

Le programme scientifique consistera en un choix de sujets d'intérêt interdisciplinaire général sur les sciences de la mer. Ce choix sera fait par le Comité du programme scientifique dont les membres seront nommés par le bureau du Comité scientifique de la recherche océanique du Conseil international des unions scientifiques.

Des informations supplémentaires seron communiquées au fur et à mesure des besoins — *Leo O'Quinn, Secrétaire, Comité national de direction, a.s. Comité canadien d'océanographie, 240, rue Sparks, Ottawa (Ontario), Canada K1A 0E6.*

Symposium on Multidisciplinary Studies on Hudson–James Bay

The symposium will be held April 28–30, 1981 at the University of Guelph, Guelph, Ont. Its objectives are to assess the current state of knowledge; report on research in the area; relate features and processes of this area to other similar arctic and subarctic zones; foster interdisciplinary interaction and determine needs for further research.

For information please write to *Professor I. P. Martini, Department of Land Resource Science, Ontario Agricultural College, University of Guelph, Guelph, Ontario N1G 2W1.*

Colloque sur les études multidisciplinaires des baies d'Hudson et James

Le colloque aura lieu du 28 au 30 avril 1981 à l'Université de Guelph, à Guelph, en Ontario. Ses objectifs sont : évaluer l'état actuel de nos connaissances; faire rapport des recherches effectuées dans la région; relier les caractéristiques et processus de cette région à ceux d'autres zones arctiques et subarctiques semblables; promouvoir l'interaction entre disciplines et identifier les besoins de recherche future.

Pour renseignements, on est prié d'écrire au *Professeur I. P. Martini, Department of Land Resource Science, Ontario Agricultural College, University of Guelph, Guelph, Ontario N1G 2W1.*

COMING NEXT MONTH / À PARAÎTRE LE MOIS PROCHAIN

Inhibition of Atlantic salmon hatching at low pH
R. H. Peterson, P. G. Daye, and J. L. Metcalfe

Genetic differences and environments of mussel populations in the maritime provinces
K. E. Gartner-Kepkay, L. M. Dickie, K. R. Freeman, and E. Zouros

Population or stock structure of lake whitefish, *Coregonus clupeaformis*, in northern Lake Michigan as assessed by isozyme electrophoresis **M. Imhof, R. Leary, and H. E. Booke**

A salicylate-hypochlorite method for determining ammonia in seawater
C. E. Bower and T. Holm-Hansen

Viral erythrocytic necrosis: some physiological consequences of infection in chum salmon
J. R. MacMillan, D. Mulcahy, and M. Landolt

Plasma cortisol levels of fingerling rainbow trout at rest, and subjected to handling, confinement, transport, and stocking **B. A. Barton, R. E. Peter, and C. R. Paulencu**

A study of *Haematractidium scombri* in Atlantic mackerel, *Scomber scombrus*
S. A. MacLean

Measurement of whole lake sediment accumulation and phosphorus retention using lead-210 dating **R. D. Evans and F. H. Rigler**

Spring transition period in Lake Ontario — a numerical study of the causes of the large biological and chemical gradients **D. Scavia and J. R. Bennett**

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R. J. Naiman and J. R. Sedell

Dynamics of selenium in mercury-contaminated experimental freshwater ecosystems
J. W. M. Rudd, M. A. Turner, B. E. Townsend, A. Swick, and A. Furutani

Stress responses to transportation and fitness for marine survival in coho salmon (*Oncorhynchus kisutch*) smolts **J. L. Specker and C. B. Schreck**

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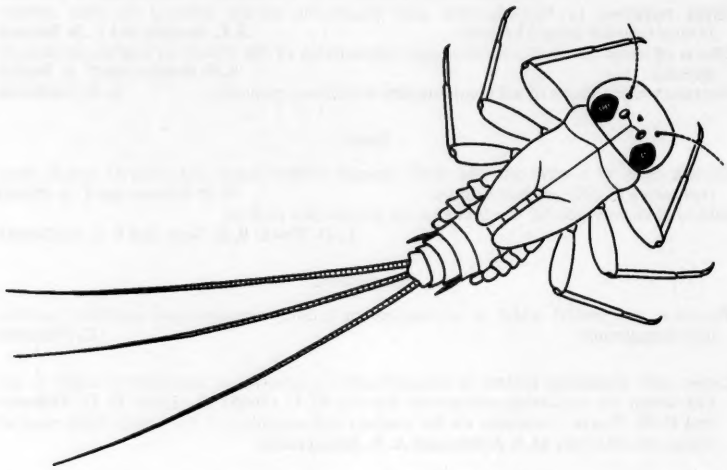
BULLETIN 201

Biosystematic Revision of the Genus *Stenonema* (Ephemeroptera: Heptageniidae)

A. F. BEDNARIK AND W. P. MCCAFFERTY

(73 p., Cat. no. Fs 94-201; Canada $8.50, other countries $10.20)



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Canadian Journal of Fisheries and Aquatic Sciences

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(Anciennement le *Journal de l'office des recherches sur les pêcheries du Canada*)

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